



# World Aquaculture 2017

**Sustainable Aquaculture  
New Frontiers for Economic Growth  
Spotlight on Africa**

**June 26-30, 2017**

**Cape Town International Convention Centre  
Cape Town, South Africa**

**The Annual International Conference & Exposition of  
World Aquaculture Society**

Hosted by

Aquaculture Association of Southern Africa

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# WELCOME

Welcome to World Aquaculture 2017 - the first international conference and exposition of the World Aquaculture Society (WAS) to be held on the African continent. This year's annual meeting is co-organized with the Aquaculture Association of Southern Africa (AASA) and the South African Department of Agriculture, Forestry and Fisheries (DAFF). World Aquaculture 2017 has attracted wide sponsorship and support from African development institutions including the South African Department of Agriculture, Forestry and Fisheries, the African Union and NEPAD, and the WorldFish Centre. On behalf of the World Aquaculture Society, its chapters and our co-hosts, it is our pleasure to welcome you to Cape Town, South Africa. We hope you enjoy and benefit from the conference, the trade show, and spend time enjoying the culture, history and incredible natural beauty that Cape Town has to offer.

The conference theme, "Sustainable Aquaculture – New Frontiers for Economic Growth – Spotlight on Africa" highlights the potential of aquaculture production to support economic development and investment opportunities in Africa - the world's second fastest growing regional economy. We are pleased to present a five-day program entitled "Spotlight on Africa" that includes an opening ceremony plenary address by Guinean President Alpha Condé, Chairman of the African Union and African Union special ambassador for aquaculture. On the first full-day of the conference, two plenary addresses will be given: Dr. Rohanna Subasinghe, former chief of Aquaculture for FAO, will speak on "Feeding the Nine Billion: the Role of Aquaculture" followed by Dr. Sloans Chimatiro, Acting Country Director with WorldFish Zambia, speaking on "African Perspectives on Aquaculture". In recognition of the early developmental stage of African Aquaculture, special sessions on Developing African Aquaculture Value Chains, Financing African Aquaculture and African Aquaculture Policy will be organized by the African Union, NEPAD, World Bank and WorldFish. Our trade show will feature over 90 exhibitors and the WAS Scientific Program at the conference features 70 sessions that will be conducted over four days, covering a diverse array of aquaculture-related topics and speakers, as well as a dedicated poster session.

Representing the coming of age of African aquaculture and a significant milestone for the global aquaculture community, WAS will host a meeting to launch the formation of a WAS Africa Chapter on Tuesday afternoon following the Spotlight on Africa program. Be sure to join us for this important organizational to form the African Chapter of WAS.

For students, we will host a special tour of the DAFF Marine Research Aquarium, the Student Spotlight Presentations and awards, special seminars, and the student reception and a social event. Of course there will be many social events (Welcome reception, Student reception, President's reception, and Happy hours) that are important occasions to network with new and old friends from around the world. Be sure to participate in the Farm tours, which will highlight South Africa's Aquaculture Industry. On behalf of the World Aquaculture Society, we want to thank and recognize the Steering and Program Committee members for the many years of work that went into organizing this event. Best wishes for a productive and enjoyable conference.

Kevan L. Main, Roger Krohn & Siphokazi Ndudane – WA17 Steering Committee Co-Chairs  
Maria Haws, Peter Britz & Michael Schwarz – WA17 Program Committee Co-Chairs



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# World Aquaculture 2017

## ABSTRACTS



## EFFECTS OF COMBINED PHYTOGENIC COMPOUNDS ON GROWTH AND NUTRITIONAL PHYSIOLOGY OF NILE TILAPIA *Oreochromis niloticus*

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Phytogenics, plant products that can be in form of ground herbal parts or essential oils extracted from plants, are among feed additives being evaluated in fish diets to enhance fish growth and feed efficiency. Phytogenic products contain various bioactive compounds at different concentrations, with those at higher concentrations primarily determining biological properties of phytogenics. In our previous research, phytogenic compounds limonene and thymol, major constituents of the plants citrus and thyme respectively, were found to have growth-promoting effects in Nile tilapia (*Oreochromis niloticus*). It is argued that combinations of some phytogenic compounds might have stronger growth-promoting effects than their individual compounds. Moreover, the mode of action of phytogenics in enhancing fish growth is not well understood especially with respect to nutritional physiology. This study was therefore carried out to investigate the effects of a dietary combination of limonene and thymol, compared with each compound individually, on the growth and nutritional physiology of Nile tilapia.

Nile tilapia of  $1.6 \pm 0.0$  g (mean  $\pm$  standard error) were fed a commercial diet supplemented with 0 ppm limonene and thymol (control), 400 ppm limonene (L), 500 ppm thymol (T) and a combination of 400 ppm limonene and 500 ppm thymol (LT) for nine weeks. Each treatment was tested in quadruplet tanks, each tank stocked with 38 fish. Growth performance and physiological pathways previously identified to respond to the phytogenic compounds were investigated by analysing the expression of some marker genes within pathways of somatotrophic axis-mediated growth (insulin growth factor I - *igf-I* and growth hormone - *gh*), nutrient digestion, absorption and transport (mucin-like protein - *muc*, oligo-peptide transporter 1 - *pept1* and phospholipase A2 - *pla2*), and lipid metabolism (lipoprotein lipase - *lpl*, sterol regulatory element binding transcription factor 1 - *srebfl* and alkaline phosphatase - *alp*).

Diets L and LT significantly enhanced final weight and feed efficiency of the fish to similar extents compared with the control. Although diet T increased final fish weight, it was not statistically higher than the control. The results indicate that limonene was the major contributor towards the enhanced fish growth suggesting lack of synergistic or additive effects of the combined compounds on somatic growth. The expression of *igf-I*, *muc*, *pept1* and *lpl* in Nile tilapia was significantly increased by diet LT compared to the control, and diet LT showed generally no statistical differences with diets L or T (Table 1).

*Igf-I* regulates somatic growth with high expression of *igf-I* associated with increased growth. *Muc* stimulates mucus secretion in the intestine lubricating the gut and aiding nutrient translocation. *Pept1* aids protein absorption and *lpl* is involved in lipid uptake which can spare protein for growth. Overall, the gene expression of the biomarkers for nutrient digestion, absorption and transport (*muc*, *pept1*), lipid metabolism (*lpl*), and somatotrophic axis growth (*igf-I*) did not indicate synergistic or additive effects of limonene and thymol in Nile tilapia. The results provide insights for formulating growth-promoting feeds for Nile tilapia supplemented with both limonene and thymol or only limonene.

**Table 1:** Relative expression of *Igf-I*, *gh*, *lpl*, *alp* and *srebfl* in the liver and *muc*, *pept1* and *pla2* in the fore-intestine of Nile tilapia fed on diets containing either limonene, thymol or their combination for nine weeks. Means ( $\pm$  standard error) in a row with different letters are statistically different ( $P < 0.05$ ).

| Gene          | Relative gene expression level |                    |                    |                 | P value |
|---------------|--------------------------------|--------------------|--------------------|-----------------|---------|
|               | control                        | L                  | T                  | LT              |         |
| <i>igf-I</i>  | $1.0 \pm 0.3^a$                | $2.7 \pm 0.5^b$    | $2.2 \pm 0.7^{ab}$ | $3.3 \pm 0.7^b$ | 0.025   |
| <i>gh</i>     | $1.0 \pm 0.3$                  | $1.3 \pm 0.2$      | $1.1 \pm 0.2$      | $1.4 \pm 0.2$   | NS      |
| <i>lpl</i>    | $1.0 \pm 0.1^a$                | $1.9 \pm 0.3^{ab}$ | $1.4 \pm 0.3^a$    | $3.9 \pm 0.9^b$ | 0.003   |
| <i>alp</i>    | $1.0 \pm 0.1$                  | $1.0 \pm 0.1$      | $0.8 \pm 0.1$      | $0.9 \pm 0.1$   | NS      |
| <i>srebfl</i> | $1.0 \pm 0.3$                  | $1.3 \pm 0.2$      | $1.5 \pm 0.2$      | $1.6 \pm 0.2$   | NS      |
| <i>muc</i>    | $1.0 \pm 0.1^a$                | $1.8 \pm 0.1^{ab}$ | $1.6 \pm 0.3^{ab}$ | $2.1 \pm 0.2^b$ | 0.025   |
| <i>pept1</i>  | $1.0 \pm 0.1^a$                | $1.5 \pm 0.1^{ab}$ | $1.4 \pm 0.2^{ab}$ | $1.7 \pm 0.1^b$ | 0.047   |
| <i>pla2</i>   | $1.0 \pm 0.1$                  | $1.3 \pm 0.1$      | $1.2 \pm 0.1$      | $1.4 \pm 0.2$   | NS      |

L, 400 ppm limonene; T, 500 ppm thymol; LT, 400 ppm limonene and 500 ppm thymol.

## MASS MORTALITIES ASSOCIATED WITH ISOPODA INFESTATION AS A RESULT OF UNCONTROLLED BIOSECURITY MEASURES IN LAKE QARUN, EGYPT

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Crustacean parasitic infestations are associated with high fish mortalities and economic losses in aquaculture. Conventional chemical treatments to control these infestations adversely affect fish and aquatic environment. Therefore, finding alternative control measures through vaccination or environment-friendly approaches is currently a global concern.

Lake Qarun is a closed lake covers an area of 235 km<sup>2</sup>, and stretches for 40 km from east to west (Fig. 1). Water in the lake was originally fresh but is currently salty and turbid.

The present study aims to investigate the main reason behind the appearance of unusual external parasite heavily infesting all fish species and causing mass fish mortalities in Lake Qarun, Fayoum province, Egypt during the summer season of 2015.

A total number of 300 fish of three fish species *Tilapia zilli*, *Mugil cephalus* and *Solea vulgaris* (100/ each species) were collected and transported alive to the Faculty of Veterinary Medicine, Mansoura University, Egypt for clinical, parasitological, and pathological examination. In addition, water samples were also collected for the physicochemical analysis and measuring the concentration of trace element in water.

Parasitological results revealed the existence of two different types of isopod species identified as *Nerocila orbignyi* and *Renocila thresherorum* heavily infesting the branchial cavity of all the collected fish (fig.2). The gills showed sever congestion, edema and deformity of the gill arch (data not shown).

Water analysis (table 1) revealed higher salinity levels that enabled the unusual acclimatization of the infesting parasites. In addition, total dissolved Fe, Cd, Cu and Pb were higher than the permissible limits. Finally, after investigating the main reason behind the presence of such parasites in this closed Lake, we concluded that it was due to a breach in the biosecurity measures during the routine fry transfer to the lake.

Currently, fishing in the lake is totally prohibited by the government and the lake is almost free of fish that has a huge economic loss on the aquaculture industry.

Some control measures are currently being adopted trying to solve that problem in such an important Lake. In addition, we are trying to find a suitable measure to control parasite through detecting its virulence gene to be used as tool for vaccination strategy to limit its spreading in other fish farms or other lakes in Egypt.

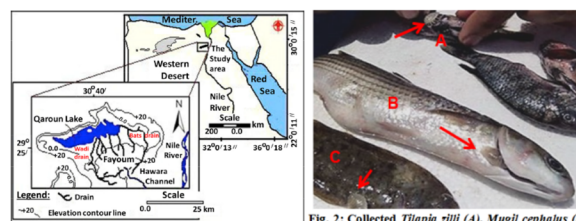


Fig. 1: Location map of Lake Qarun in Egypt with its relevant drain according to El-Sayed et al., 2015

Fig. 2: Collected *Tilapia zilli* (A), *Mugil cephalus* (B) and *Solea vulgaris* (C) infested with isopoda (red arrow) collected from Lake Qarun

Table (1): Physicochemical analysis data of the collected water samples

| Variable                         | Mean±SD   |
|----------------------------------|-----------|
| Temperature °C                   | 30.3±0.05 |
| Dissolved Oxygen (mg/L)          | 7.8±0.17  |
| PH                               | 8.1±0.30  |
| Salinity (g/L)                   | 40.2±0.11 |
| Biochemical Oxygen Demand (mg/L) | 8±0.31    |
| Chemical Oxygen Demand (mg/L)    | 12±0.18   |
| TDS (mg/L)                       | 2460±0.02 |



## EFFECTS OF DIFFERENT PLANT OILS ON GROWTH AND FATTY ACID COMPOSITION OF JUVENILE OREOCHROMIS NILOTICUS

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Research on fish oil replacements has become critical because of dwindling marine catches globally and the need to address sustainability issues in feed production; it has become important that alternative sources are identified that do not compromise the health benefits by altering the fatty acid profile of the fish, thus reducing the EFAs. Additionally, there have been concerns about the low levels of omega-3 fatty acids in cultured tilapia; more needs to be done to improve the fatty acid composition of this widely consumed and cheap protein source..

An 8-week trial was conducted to investigate the effect of five (5) experimental diets (iso-nitrogenous and iso-caloric from three different plant oils) and a commercial diet used as a control (D1) on the growth performance and muscle fatty acids of *Oreochromis niloticus*. The five diets were: 100%Linseed oil (D2), 100% Soy oil (D3), 1Linseed: 1Soy (D4), 1Linseed: 1Palm (D5) and 1Soy:1Palm (D6). All male fingerlings of average weight  $28 \pm 4.0$ g were stocked at 25/hapa; each treatment had four replicates. Fish were fed at 3% body weight twice daily.

Fishes fed with the experimental diets had a slightly reduced growth compared to the control, but with no differences among the experimental diets. There was an indication that high amounts of n-3 depressed growth in tilapia. The n-3 content in fish muscle at the end of the study ranged from a low of 3.5% in D1 to a high of 22.4% in D2. Fish fed diets containing linseed oil had the highest amount of n-3 (22.4%) which was statistically significantly higher than all the other experimental diets. The dominant n-3 was the ALA typical of plant oils; conversion to longer chain EPA and DHA was not very effective possibly due to the short duration of the trial.

This study shows although growth performance of the experimental diets were lower than that of the control, the Nile tilapia has the ability to retain fatty acids from feed into muscles for the benefit of the consumer.

## PERCEPTION OF FISH FARMERS ON CLIMATE CHANGE IN AFRICA: (THE CASE OF NIGERIA AND EGYPT)

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Empirical observations and climate models both indicate that global climate and ocean conditions have been changing over the last 100 years and will likely change more rapidly in the future. This study examined the perceptions of fish farmers in rainfed (blue water) and irrigated (green water) aquaculture in Africa. South West Nigeria and Egypt were considered as rainfed and irrigated fish farms/aquaculture respectively. Both primary and secondary data were used. Multistage sampling technique was used. The countries were purposively selected because they represent good examples of rainfed and irrigated aquaculture in Africa. Three States were randomly selected in South West Nigeria while in Egypt the study area were purposively selected from the governorates concentrating mainly on the regions where aquaculture is practice. KoboCollect method was used to design relevant questions on the fish farmers' socioeconomic characteristics, production activities, marketing, labour services and costs of production, perception on climate change among others. KoboCollect was also used to analyse and interpret the results of the entries. Interview schedule and Focus Group Discussions (FGD), were also employed to get necessary information from the respondents on. Data on climate variables were obtained from reanalyzed satellite data using Google Earth Explorations (GEE).

The result of the socio-economic characteristics of the respondents revealed that 70% of the fish farmers were in the active and productive age bracket of 26-55 years, 92% were male, 83% were married, and 64% had their level of education above secondary school. The mean and standard deviation of the number of person in the household were 4 and 1.6 respectively. In Nigeria, 61% of the respondents rely on stream and river for their source of water and 54% had other occupation apart from taking aquaculture as their major occupation. Fish production and supply in the area could not meet up with the demand of the consumers as a result of financial constraints, low capital investment, high cost of fish stocking and feeding, dry season and pollution, only 32% were ready to take precautionary measures. Although, 93% of them have the ideal of climate change, 64% believe that the change will linger and persevere in the next 10-20 years. Google Earth Explorations (GEE) using the coordinates of the various study sites showed the trends of climate change in the study area. Egypt is the only African country to have achieved the scale of aquaculture expansion observed elsewhere in the world. However, efforts should be geared toward regional integration in order to encourage aquaculture practices in other parts of Africa and climate change investment should be encouraged.

**Acknowledgement:** African Women in Agriculture Research and Development (AWARD) and World Fish Centre, Egypt



## **POTENTIALS OF CAPTURE BASED AQUACULTURE (CBA) IN THE COASTAL REGIONS OF ONDO STATE, NIGERIA**

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Acknowledgement: The World Academic of Sciences (TWAS) - African-German Network of Excellence  
Sciences (AGNES) 2015 Young Scientists Grant

This study evaluates the potentials of Capture Based Aquaculture (CBA) in the coastal regions of Ondo State, Nigeria. The study specifically examined the socio-economic characteristics of capture based fisherfolks in coastal regions of Ondo State, it determined the level of awareness, perception and percentage of the fisherfolks that practice CBA. Prospects and problems encountered by capture based fisherfolks in the study area were also investigated. A multistage sampling technique was used to select one hundred (100) respondents who were fishermen/women in the study area and structured questionnaires were administered. The method of data analysis included descriptive statistics and likert scale. Fishing has become a way of life and primary occupation of the coastal dwellers who depend on it for their livelihood and sustenance.

The study revealed that only 13% of the respondents were into Capture Based Aquaculture (CBA) for family consumption, 26% embarked on CBA for income, the rest (61%) combined both purposes (consumption and income). Inadequate funds, high cost of fishing gears, pollution of water by petroleum mistreatment, poor fishing technology, weather and climate adverse effect on sea level, poor market among others were problems faced by the fisherfolks in the study area. The study concluded that CBA provided economic opportunities for coastal regions of Ondo State. The study therefore recommends empowering the operators of CBA with subsidized inputs and provision of funds either in kind/cash to encourage more fisherfolks to practice CBA in the coastal regions of Ondo State, Nigeria.

## **BIOECONOMIC FEASIBILITY OF AQUAPONICS FOR THE SUSTAINABLE DEVELOPMENT OF FRESHWATER FIN FISH AQUACULTURE IN SOUTH AFRICA**

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Aquaculture is an emerging industry in South Africa with annual production less than 7,000 tonnes and is therefore, considered an underdeveloped sector. Freshwater fish culture is severely limited by the supply of suitable water as South Africa is a dry country and currently being confronted with water supply crisis due to combination of low rainfall, high evaporation rates, expanding economy and growing population whose geographical demands for water do not conform to the distribution of exploitable water supplies. The challenge for South Africa with heavy utilisation of the nation's water resources and the limited availability of further supplies lies in the efficient and balanced use of water by all sectors to create a conducive and healthy socioeconomic environment.

Aquaponics as a leapfrog technology offers South Africa the opportunity and leverage to grow its aquaculture industry rapidly to become a key player globally, if identified impediments such as weak government involvement, stringent and unfavourable policies as well as dearth of skilled resources are critically and strategically tackled by considering inputs from all industry stakeholders. Leapfrog technology has the potentials of addressing key challenges (i.e. climate, water, land and food security) confronting the growth of aquaculture in South Africa. The aim of this study therefore, is to assess the viable technical, biological, and socioeconomic prospects for the development of sustainable freshwater aquaculture cum organic vegetables production in South Africa using aquaponics as a leapfrog technology.

Aquaponic systems were designed to compare feasibility of food fish (*Oreochromis mossambicus* and *O.niloticus*) and ornamental fish koi (*Cyprinus carpio*) integrated with both gravel bed and floating raft plant growth media for the production of leafy vegetable (lettuce and spinach) and medicinal herb (basil). A generic financial model will be designed using Microsoft excel program to integrate biological parameters and financial variables to determine the feasibility of each aquaponic system which can be scaled and adapted for aquaponic operations. Comprehensive market study and skill needs analysis of the industry will be conducted to determine the sector's strength, weakness, opportunity and strength (SWOT) with aim of developing a growth strategy. Results obtained from this study will assist with the formulation of favourable policies and legislations that contribute to the rapid development of freshwater finfish aquaculture in South Africa.



## GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND UTILIZATION OF AFRICAN CATFISH FED DIETS SUPPLEMENTED WITH *Tamarindus indica* L AS DIETARY FEED ADDITIVE

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A feeding trial was conducted with 900 *Clarias gariepinus* fingerlings (initial mean weight  $5.75 \pm 0.01$ g) in 100litre tanks with 30 fingerlings per tank in triplicates in an indoor static renewal system. Fish were fed experimental diets supplemented with 0.0% (positive control), 2g oxytetracycline (OTC)/kg diet (negative control), four varying levels (5, 10, 15 and 20g/kg diets) each of Tamarind Pulp (TP) and Leaf (TL) at 3% body weight daily for 12 weeks. Weight Gain (WG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Nitrogen metabolism (Nm) and Apparent Nutrient Digestibility (ADC) were calculated.

*C. gariepinus* fed diets treated with TP and TL had significantly higher ( $P < 0.05$ ) WG, PWI, SGR, Nm, and lower FCR than those fed with the control diets. All tamarind-treated groups exhibited significantly higher ( $P < 0.05$ ) PER and FPI while the fish fed 20g TP/kg and 10gTL/kg diets had the highest significant survival rate compared to the control groups. The highest PER were obtained from fish fed with 10g TP and TL/kg diets. The ADC of protein of diet treated with  $\geq 15$ g TP and  $\geq 10$ g TL/kg diets were significantly higher than OTC-treated group. Significantly lower incidence of cost and higher profit index were exhibited by *C. gariepinus* fed diets containing 10g TP and TL/kg diets. Supplementation of *C. gariepinus* diets with TP and TL significantly enhanced growth performance and nutrient utilization.

Table 1: Gross composition (g/kg dry matter) of experimental diets (40% crude protein) at varying inclusion of tamarind pulp and leaf

| Ingredients                    | Diets (g/kg) |        |       |       |       |       |       |       |       |       |
|--------------------------------|--------------|--------|-------|-------|-------|-------|-------|-------|-------|-------|
|                                | 0.00         | 2.0OTC | 5TP   | 10TP  | 15TP  | 20TP  | 5TL   | 10TL  | 15TL  | 20TL  |
| Fish meal                      | 241.1        | 241.1  | 241.1 | 241.1 | 241.1 | 241.1 | 241.1 | 241.1 | 241.1 | 241.1 |
| SBM                            | 300.0        | 300.0  | 300.0 | 300.0 | 300.0 | 300.0 | 300.0 | 300.0 | 300.0 | 300.0 |
| Cr <sub>2</sub> O <sub>3</sub> | 5.0          | 5.0    | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   |
| Others                         | 453.9        | 451.9  | 448.9 | 443.9 | 438.9 | 433.9 | 448.9 | 443.9 | 438.9 | 433.9 |

Table 2: Growth performance and digestibility of nutrients of *C. gariepinus* fed Tamarind treated diets

| Diets (g/kg) | WG(g)                         | FCR                           | PER                             | ADC CP (%)                    | Incidence of cost                |
|--------------|-------------------------------|-------------------------------|---------------------------------|-------------------------------|----------------------------------|
| 0.00         | 24.97 $\pm$ 3.79 <sup>d</sup> | 1.82 $\pm$ 0.07 <sup>a</sup>  | 1.38 $\pm$ 0.06 <sup>e</sup>    | 52.98 $\pm$ 6.54 <sup>e</sup> | 327.18 $\pm$ 13.39 <sup>a</sup>  |
| 2.0 OTC      | 24.34 $\pm$ 1.28 <sup>d</sup> | 1.81 $\pm$ 0.07 <sup>a</sup>  | 1.38 $\pm$ 0.05 <sup>e</sup>    | 62.83 $\pm$ 0.11 <sup>d</sup> | 332.26 $\pm$ 12.49 <sup>a</sup>  |
| 5.0TP        | 28.90 $\pm$ 2.21 <sup>c</sup> | 1.61 $\pm$ 0.03 <sup>b</sup>  | 1.55 $\pm$ 0.03 <sup>d</sup>    | 58.54 $\pm$ 1.31 <sup>d</sup> | 290.64 $\pm$ 5.69 <sup>b</sup>   |
| 10.0TP       | 35.29 $\pm$ 0.97 <sup>b</sup> | 1.51 $\pm$ 0.07 <sup>bc</sup> | 1.66 $\pm$ 0.08 <sup>a</sup>    | 61.68 $\pm$ 0.99 <sup>d</sup> | 272.13 $\pm$ 13.52 <sup>bc</sup> |
| 15.0TP       | 36.80 $\pm$ 1.58 <sup>b</sup> | 1.52 $\pm$ 0.02 <sup>bc</sup> | 1.65 $\pm$ 0.02 <sup>ab</sup>   | 68.51 $\pm$ 3.09 <sup>c</sup> | 273.19 $\pm$ 3.40 <sup>bc</sup>  |
| 20.0TP       | 40.11 $\pm$ 1.22 <sup>a</sup> | 1.52 $\pm$ 0.03 <sup>bc</sup> | 1.64 $\pm$ 0.03 <sup>abcd</sup> | 83.09 $\pm$ 2.44 <sup>a</sup> | 275.75 $\pm$ 5.55 <sup>bc</sup>  |
| 5.0TL        | 28.69 $\pm$ 2.42 <sup>c</sup> | 1.61 $\pm$ 0.05 <sup>bc</sup> | 1.56 $\pm$ 0.05 <sup>cd</sup>   | 53.57 $\pm$ 6.09 <sup>e</sup> | 289.90 $\pm$ 8.54 <sup>bc</sup>  |
| 10.0TL       | 36.72 $\pm$ 1.92 <sup>b</sup> | 1.50 $\pm$ 0.02 <sup>c</sup>  | 1.66 $\pm$ 0.02 <sup>a</sup>    | 78.89 $\pm$ 1.03 <sup>a</sup> | 271.33 $\pm$ 3.41 <sup>c</sup>   |
| 15.0TL       | 31.53 $\pm$ 3.07 <sup>c</sup> | 1.60 $\pm$ 0.09 <sup>bc</sup> | 1.57 $\pm$ 0.09 <sup>bcd</sup>  | 68.52 $\pm$ 1.03 <sup>c</sup> | 289.47 $\pm$ 15.92 <sup>bc</sup> |
| 20.0TL       | 35.71 $\pm$ 1.12 <sup>b</sup> | 1.52 $\pm$ 0.02 <sup>bc</sup> | 1.65 $\pm$ 0.02 <sup>abc</sup>  | 73.83 $\pm$ 2.31 <sup>b</sup> | 274.68 $\pm$ 3.68 <sup>bc</sup>  |

## ECONOMIC EFFICIENCY OF FISH FARMING TECHNIQUES IN NIGERIA: ARE FARMERS REALLY EFFICIENT?

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The study examined the efficiency of various fish farming techniques employed by 590 fish farmers in Nigeria with particular reference to production systems, rearing methods, and water retention capacity. It specifically identified the typology of fish farming and the economic efficiency of various techniques using stochastic frontier production to determine the efficiency of fish farmer in resources use.

Estimation of the stochastic frontier production functions on the most profitable techniques showed that the mean technical efficiency level was 98.0 % for flow through system (FTS) and water recirculatory with  $t_c = 4.441$  indicating significant, while it was 85.1 % for earthen pond water. Allocative efficiencies were 0.43 for FTS while water recirculatory systems were 0.46 for Nigeria type and 0.55 for Dutch system. Allocative efficiency in earthen pond was 1.0. Figures obtained for the mean economic efficiency were 0.91; 0.43; 0.46 and 0.55 for earthen pond, FTS, and water recirculatory for Nigeria and Dutch systems respectively. Based on profitability criterion only, for water exchange capacity, flow technique (10.6) was better than stagnant; on rearing techniques, plastic tank (6.2) was the best; on culture systems, water recirculatory (7.12) had the highest; on types of fish feed, floating feed (6.13) was better than local. The study revealed that farmers were technically sound in input combination but the high cost of fish feed, quality of fish seed, energy, enabling and marketing were identified as barrier to the sustainable growth of aquaculture in Nigeria.

Aquaculture is the fastest growing food production sector in the world, with an annual growth rate of 8%, and currently supplies 50% of total fish and shellfish for human consumption. Demand and price for aquatic products are projected to continue rise. With an annual production of ~300,000 metric tonnes (FDF, 2015), the Nigerian aquaculture industry is relatively small by global standards – but this not need to be. Investment in R&D that addresses the technological bottlenecks to production - including new species- husbandry and reducing the sector's environmental footprint. This would improve Nigeria's reputation and services for aquaculture technology and respond to the increasing global demand for high quality seafood.

**Table 1: Economic analysis of Fish Farming Techniques in Lagos State Nigeria**

| Economic indices   | Stagnant                 | FTS                      | WRSN                      | WRSD                     | Overall mean | F       |
|--------------------|--------------------------|--------------------------|---------------------------|--------------------------|--------------|---------|
|                    | Mean                     |                          |                           |                          |              |         |
| Output (Kg)        | 7,527.0 <sup>b</sup>     | 22,750.0 <sup>a</sup>    | 23,216.0 <sup>a</sup>     | 26,907.0 <sup>a</sup>    | 19,025.92    | 38.29*  |
| Yield (Kg/ha)      | 21,611.0 <sup>b</sup>    | 60,597.0 <sup>a</sup>    | 59,811.0 <sup>a</sup>     | 54,949.0 <sup>a</sup>    | 46,911.02    | 8.74*   |
| Revenue (₦)        | 2,374,423 <sup>b</sup>   | 9,638,440 <sup>a</sup>   | 8,496,500 <sup>a</sup>    | 9,110,141 <sup>a</sup>   | 6,930,581    | 19.35*  |
| Variable cost (₦)  | 660,273.0 <sup>b</sup>   | 1,742,991.0 <sup>a</sup> | 1,432,392.0 <sup>a</sup>  | 1,781,235.0 <sup>a</sup> | 1,326,644    | 16.25*  |
| Depreciation (₦)   | 73,666.0 <sup>b</sup>    | 83,818.0 <sup>b</sup>    | 133,480.0 <sup>a</sup>    | 142,994.0 <sup>a</sup>   | 107,379.7    | 4.58*   |
| Total cost (₦)     | 733,939 <sup>b</sup>     | 1,826,809 <sup>a</sup>   | 1,565,873 <sup>a</sup>    | 1,924,229 <sup>a</sup>   | 1,434,024    | 16.92*  |
| Profit (₦)         | 1,973,401.0 <sup>c</sup> | 6,082,540.0 <sup>b</sup> | 6,515,937.0 <sup>ab</sup> | 7,572,918.0 <sup>a</sup> | 5,243,270    | 29.73*  |
| Profitability      | 4.40 <sup>b</sup>        | 6.90 <sup>a</sup>        | 6.58 <sup>a</sup>         | 7.12 <sup>a</sup>        | 6.08         | 10.81*  |
| <i>Efficiency:</i> |                          |                          |                           |                          |              |         |
| Technical          | 0.85 <sup>b</sup>        | 0.98 <sup>a</sup>        | 0.98 <sup>a</sup>         | 0.98 <sup>a</sup>        | 0.94         | 53.18*  |
| Allocative         | 1.00 <sup>a</sup>        | 0.43 <sup>c</sup>        | 0.46 <sup>c</sup>         | 0.55 <sup>b</sup>        | 0.64         | 148.14* |
| Economic           | 0.91 <sup>a</sup>        | 0.43 <sup>c</sup>        | 0.46 <sup>c</sup>         | 0.55 <sup>b</sup>        | 0.61         | 69.93 * |

# AQUACULTURE VALUE CHAIN IN NIGERIA: EMERGING TRENDS

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## Introduction

Aquaculture is playing an increasingly important role in global food production and food security. It is the fastest growing food production sector in the world, with an annual growth rate of 7%, and now supplies 50% of total fish and shellfish for human consumption. Although the aquaculture industry is growing, Nigeria currently imports 60% of its seafood. There is an opportunity for a step-change in the sustainable growth of Nigeria aquaculture. This would increase Nigeria's self-sufficiency in seafood and enable the industry to respond to the increasing demand for high quality seafood.

## Objective of the study

The study aimed at mapping out the value-chain for farmed fish in order to describe the main stakeholders and the flow of product; understand the costs and earnings profiles of the different sub-sectors of the value-chain and identify the key constraints and problems impacting on different actors in the value-chain.

## Research methodology

The sampling technique has been done to incorporate all the stakeholders in order to produce a complete picture of the aquaculture value chain. The study employs an integrated value chain analysis (IVCA). It is a channel mapping methodology of tracing a product flow through an entire channel from the point of product conception to the point of consumption. This process highlights the underlying patterns of inputs, constraints, and competitive advantages that a producer has. It also traces the path of all value adding and non-value-adding activities associated with the production of a good and approximates costs involved at each stage. It looks at policy, institutions, markets, infrastructure, access, and representation issues that can influence the opportunities and constraints of actors in the value chain.

## Results and discussion

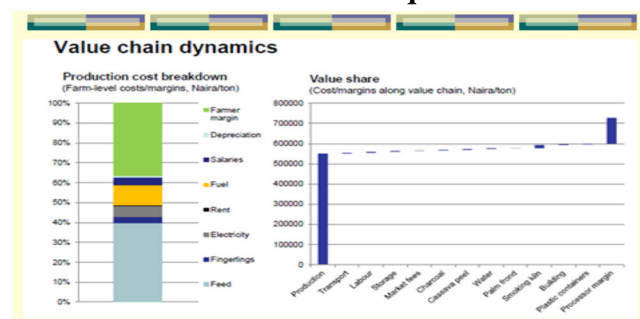
To enhance price competitiveness in aquaculture in Nigeria, there is need for substantial productivity increases to lower production costs. The first priority to increase the price competitiveness is to reduce on-farm production costs. These costs represent the largest cost-component (70% of current production. Local production costs are substantial, particularly in view of high operation costs and low yields. Low-cost fish feed and labor-saving technology offer particular promise to lower production costs.

## Conclusion and Recommendation

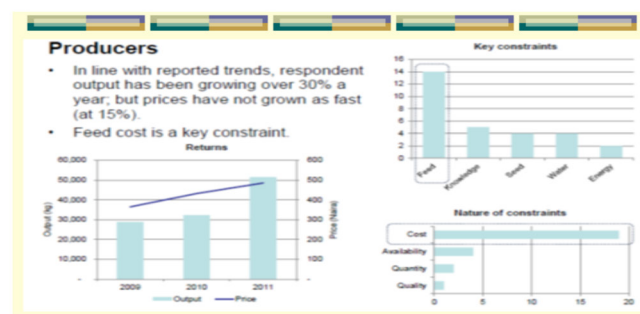
The three priority and complementary interventions needed to develop the Nigerian fish sector are - increasing efficiency along the commodity chain; improving quality management along the commodity chain and creating an enabling environment for these changes to happen.

**Acknowledgement:** The Management of the Institute for sponsoring the project.

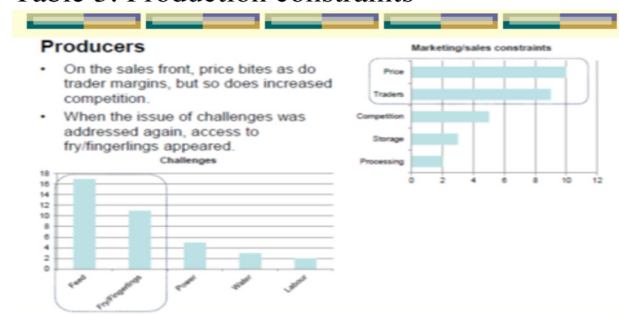
**Table 1: Production cost in aquaculture**



**Table 2: Relationship between productivity and price**



**Table 3: Production constraints**





## NEW FRONTIERS FOR ECONOMIC GROWTH – SPOTLIGHT ON NIGERIA AQUACULTURE INDUSTRY

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Aquaculture is widely recognised and promoted as a source of income, employment and food supply hence its potential as a tool for development in developing nations including Nigeria. A mapping study of operators in Lagos and Ogun States aquaculture industry was conducted. A total of 5,103 operators (hatchers and farmers) were enumerated and characterised. The industry was dominated by African catfish production, mainly operated by male (81.6% and 92.3% in Lagos and Ogun States respectively). Catfish table-size operation was the main engagement (53.8% and 48.4% in Lagos and Ogun States respectively) in the industry, followed by hatchery operation (20.7% and 35.11% in Lagos and Ogun States respectively) with few operators engaged in enterprise mix. Borehole and river/ stream are the main sources of water for operations. Large proportion of the farmers utilised feed from more than a source (imported and locally-produced) for production. Operations are mainly financed from personal savings (88.27% and 85.5% in Lagos and Ogun States respectively). Cost of feed was the largest recurrent production cost accounting for more than 60% in table-size operation. The most common sales terms were cash on delivery. High cost of feed input and insufficient fund and/ or lack of access to fund are perceived to be the most severe constraints to aquaculture production. Therefore, to further tap the potential of the Nigeria aquaculture industry for economic growth, provision of funds and easy access to credit facilities as well as local production of affordable high quality feed requires urgent attention.

## UNDER OPTIMUM REARING CONDITION, NILE TILAPIA *Oreochromis niloticus* DOES NOT MARKEDLY BENEFIT FROM COSTLY EXOGENOUS ENZYME SUPPLEMENTATION

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A study was carried out to evaluate the effects of selected exogenous digestive enzymes on Nile tilapia fed a practical diet. Tilapia (29.52±0.3g) were fed one of experimental diets supplemented with phytase (75 mg kg<sup>-1</sup>), protease (300 mg kg<sup>-1</sup>), xylanase (250 mg kg<sup>-1</sup>) from commercial sources and a control diet. Tilapia were fed at 3 % biomass per day in triplicate tanks per treatment for 8 weeks. There was no significant difference observed in percentage weight gain of tilapia fed each of the experimental diets. Similarly, the final body weight (FBW) of tilapia fed phytase (137.09±2.59 g fish<sup>-1</sup>) and protease (135.26±1.74 g fish<sup>-1</sup>) supplemented diets were not significantly different from those fed the control diet (136.42±0.41 g fish<sup>-1</sup>). However, FBW of tilapia fed the xylanase supplemented diet (14.43±0.31 g fish<sup>-1</sup>) was significantly higher ( $P < 0.05$ ) than those fed the control diet (136.42±0.41 g fish<sup>-1</sup>). There was no significant difference observed in specific growth rate, somatic indices and survival of tilapia fed the experimental diets. The dietary treatment did not have significant effects on body composition, haemato-immunological parameters and the activities of endogenous digestive enzymes of tilapia fed the experimental diets. The histological assessment of the mid-intestine sections taken from tilapia fed the experimental diets also showed no significant difference in their perimeter ratio and number of secretory goblet cells per 100 µm. It could therefore be concluded that tilapia does not benefit markedly from supplementation of a practical diet with costly exogenous digestive enzymes with respect to typical diets for this species under normal production conditions.

## **UNDER OPTIMUM REARING CONDITION, NILE TILAPIA *Oreochromis niloticus* DOES NOT MARKEDLY BENEFIT FROM COSTLY EXOGENOUS ENZYME SUPPLEMENTATION**

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## **EFFECT OF FISHMEAL REPLACEMENT WITH ANIMAL AND PLANT PROTEIN SOURCE ON GROWTH PERFORMANCE AND GUT MICROBIOTA OF DUSKY KOB, *Argyrosomus japonicus***

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Fishmeal is the major dietary protein for many farmed fish species due to its nutritional properties. However, decreasing fishmeal supply relative to demand and increasing costs threaten the sustainability and growth of the aquaculture industry. This has led to many studies on alternative proteins sources to replace fishmeal protein in aquaculture feeds.

Studies of fish nutrition have traditionally used growth performance to assess diets. However, increasing knowledge of gastrointestinal microbiome suggests that assessment of microbial communities could offer complementary information to these measurements. Therefore a multidisciplinary approach in assessing the effect of alternative proteins sources can improve our knowledge of the observed effect on growth. Hence, the aim of the present study was to investigate the effect of fishmeal replacement on growth performance and intestinal microbiota of dusky kob.

Three isonitrogenous (41 % protein) and isoenergetic (15.2 kJ/g) experimental diets were fed to dusky kob (initial average weight of  $15.7 \pm 0.4$ g) with 5 replicates and 12-fish per tank in a 60-day study. Diet 1 contained 100 % fishmeal (diet code: D100). Diet 2 was based on a mixture of animal and plant proteins. The fishmeal inclusion rate in this diet was 50 %, with protein sources being poultry meal, blood meal, soybean meal and canola meal (diet code: D50). The third diet had 0 % fishmeal inclusion rate with protein sources being blood meal and soybean meal (diet code: D0). In all the treatments crystalline essential amino acid was added to balance the essential amino acid requirement for dusky kob. The fish was fed to apparent satiation by hand twice daily. The temperature and dissolved oxygen averaged  $23.1 \pm 0.1$  °C and  $4.6 \text{ mg L}^{-1}$  and  $\text{NH}_4\text{-N}$  remained below  $0.1 \text{ mg L}^{-1}$ . At the end of the trial, fish were sampled for gut microbiota. Growth parameters were assessed; final body weight, thermal growth coefficient, specific growth rate and feed intake were assessed across diets.

The analysis of this study is in progress and data will be presented at the conference.

## **GROWTH PERFORMANCE AND SURVIVAL OF *Clarias gariepinus* LARVAE FED DECAPSULATED ARTEMIA, LIVE ARTEMIA NAUPLII AND DECAPSULATED LIVE ARTEMIA**

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Starter foods are important in the growth of *Clarias gariepinus* larvae. This research was conducted to investigate the growth and survival of African catfish (*Clarias gariepinus*) larvae fed with three (3) different types of food (dried decapsulated Artemia, live Artemia Nauplii and Decapsulated live Artemia) were evaluated for eight days in the laboratory. Larvae were obtained by artificial propagation and distributed into triplicate representing the three food type at 70 larvae per tank. The larvae were fed twice daily. At the end of the experiment, the highest survival and growth values were obtained in larvae fed decapsulated live Artemia ( $p < 0.05$ ).

## **GROWTH AND FEED CONVERSION OF NILE TILAPIA *Oreochromis niloticus* IN PONDS WITH COMMERCIAL GROW-OUT FEEDS OF VARYING PROTEIN CONTENT**

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The inability of capture fisheries to meet Ghana's demand for food fish has placed aquaculture in a pivotal role to contribute towards meeting the supply deficit. However, the high cost of commercial feeds, priced in direct proportion to crude protein content, hampers the growth of the aquaculture sector. It is hypothesized that pond-based tilapia producers, with proper fertilization, could reduce the cost of production by adopting low-cost, low-protein grow-out feeds not yet commercially available in Ghana.

The objectives of this study were to determine 1) the effect of feeds with varying crude protein levels (25%, 28% and 30%) on the growth and feed conversion ratio (FCR) of *Oreochromis niloticus*, 2) effects on pond water quality, and 3) effect on profitability of the diets used. The study was conducted on two fish farms in Ashanti Region (one public and one private), in ponds with sizes between 200m<sup>2</sup>-600m<sup>2</sup>. Ponds were fertilized before the start of the experiment and stocked at 1.8/m<sup>2</sup> with tilapia weighing between 30-60g. Fish were fed at 2-3% of their body weight throughout the study period (February-May, 2016).

Preliminary results showed no significant average effect of feed type on growth or food conversion ratio (FCR) tested across both farms. An overall FCR of 1.5-2.5 was observed. Interval FCRs were best (< 2.0) up to 150g, but increased rapidly afterwards. Stocking density, pond water depth, and stocking size were best predictors of final weight (after 80 days of grow-out) with increasing importance in that order. The FCR was significantly predicted by average pond depth and secondarily by stocking density, with shallow ponds resulting in poorer FCR. Primary production as indicated by chlorophyll *a* was higher in the farm with shallower ponds but this did not translate into higher growth or FCR. Overall, size of fish at both farms could be increased with better quality fingerlings and more successful sex reversal. However, with FCR and cost of production considerations, optimal size at harvest may be between 200-250g.



## STRUCTURAL DAMAGE IN THE KIDNEY AND LIVER OF *Clarias gariepinus* JUVENILES EXPOSED TO GRADED CONCENTRATION OF ZINC

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The damage caused in the structural formation the kidney and the liver of African catfish, *Clarias gariepinus* exposed to graded concentration of zinc was studied for a period of 96 hours. The stock solution was prepared with zinc and the fish juveniles were exposed to the following concentration in mg/L : 0.5, 0.75, 1.00, 1.25 and 1.50 with a control of 0.0mg/L where the zinc was not introduced. The regression plot of the probit value transformed mortality against the concentration of zinc showed a strong relationship with the death of the fish exposed to the concentration of the metal with  $R^2$  value of 0.8725. All the same no death was observed in the control throughout the 96 hours period of the study while the lowest death of 15% was recorded in the 0.5mg/L and the highest of 60% in the 1.50mg/L concentration of zinc. The  $LC_{50}$  was determined to be 1.25mg/L during the study time. The ultrastructural characteristics showed pathological lesion in the kidney and vacuolation of the hepatocytes of liver of the fish. The DO of the water was below the recommended level for the survival of the fish in the waters that the concentration of zinc was introduced and may cause distress to the fish. The fish showed erratic movement and distressful behaviour where they were exposed to zinc and eventually the ones that could not tolerate the toxicity metal died. The study conclude that the fish should not be exposed to the elevated concentration of zinc as it is toxic and recommend that anthropogenic activities that release zinc into the environment should be controlled.

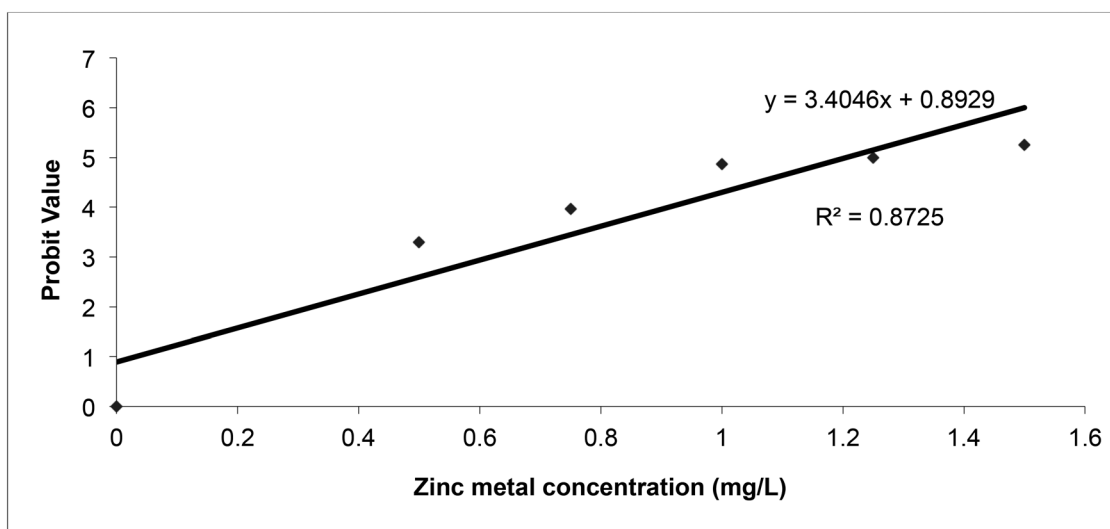


Fig 1: Regression of Probit mortality values and graded concentration of zinc exposed to juveniles of *Clarias gariepinus*.

## **ISOLATION, IDENTIFICATION, SCREENING AND EFFICACY OF POTENTIAL PROBIOTICS FROM WILD AND CULTURED SHORT-SPINE URCHIN *Tripneustes gratilla* FOR AQUACULTURE**

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The short-spined urchin, *Tripneustes gratilla* is a fast-growing, shallow water echinoid, occurring throughout the waters of the tropical Indo-Pacific. The species produces large gonads with excellent market acceptance, and is one of the most commercially important sea urchin species in countries, such as Japan. High demand has led to overfishing of natural populations prompting many countries including South Africa to explore the possibility of sea urchin aquaculture. With the increasing intensification and commercialization of aquaculture production, mass mortality during the larval stages (usually associated to the presence of opportunistic pathogens) is a major problem in the aquaculture industry. With increasing demand for environmentally friendly aquaculture, the use of probiotics is now widely accepted.

The aim of this study is to isolate, identify, screen and test the efficacy of potential probiotics from wild and cultured *T. gratilla* that could be used as potential probiotics during the early life-history stages of urchin aquaculture. This study will contribute towards the development of a protocol for the *in vitro* screening and selection of candidate probiotics which can be used in urchin culture.

## DETERMINATION OF EXPERIMENTALLY INDUCED LACTOCOCCOSIS IN CULTURED EUROPEAN SEA BASS (*Dicentrarchus labrax* L.) BY SOME DIAGNOSTIC METHODS

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Lactococcosis is a septicemic disease observed in various fish species which is caused by *Lactococcus garvieae* and it was spread to many parts of the world and causes important economic losses in aquaculture. The aim of this study is to determine the pathogenesis of experimentally induced *L. garvieae* infection in cultured European sea bass (*Dicentrarchus labrax*) and the diagnosis of the disease by using haematologic and serologic methods.

In this study, 185 European sea bass samples (50-70 g) that were supplied from a commercial fish farm located in the Aegean region of Turkey and adapted to the laboratory conditions, were used in the 50-day experimental study. After the LD<sub>50</sub> dose was determined (10<sup>8</sup> CFU/ml) two *L. garvieae* strains were injected intraperitoneally to induce infection in experiment groups.

During experimental infection; externally, scoliosis and hemorrhages and internally pale liver with nodules, expansion of the heart and spleen and hemorrhages were observed (Fig.1). In the hematologic analyses, defections on the blood cell morphology, decreases in blood parameters, erythrocyte-sedimentation rate and coagulation duration were detected. Diagnosis of the disease could be made by various serological methods (ELISA, slide and micro-well agglutination) beginning from the earlier stages and antibody level in the serum could be determined.

In conclusion, with this study it was determined that *L. garvieae* causes infections in sea bass with some significant clinical symptoms and this is the first report on the effects of this infection on the blood parameters of sea bass. It was also determined that ELISA is a more sensitive method for the diagnosis of this disease when compared to agglutination methods.



**Figure 1:** Nodule development and extension of the heart and spleen in sea bass experimentally infected with *L. garvieae*

**Acknowledgements:** This study was supported by “TÜBİTAK” (The Scientific and Technical Research Council of Turkey) with the Project number (1140766) and by “Scientific Research Projects Coordination Unit of Istanbul University” Project number (BEK-2017-24658).

## EFFECTS OF FEEDING *Momordica charantia* (BITTER MELON) LEAF DIETS ON THE REPRODUCTION OF *Oreochromis niloticus* (NILE TILAPIA)

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Tilapia is known for its ability to sexually mature at a small size (8-10 cm in body length), and at a young age (sometimes 2-3 months old) (Chapman, 2012). Fortes (2005) reported six methods of controlling tilapia populations which are expensive, not readily available (imported) and require skilled labour. Antifertility agents in many tropical plants have been used in some animal models to control fertility (Priya et.al. 2012). Kumar et.al. (2012) documented a comprehensive review of potential antifertility agents from 557 plant species, *Momordica charantia* inclusive. *Momordica charantia* (common name: bitter melon) belong to the family Cucurbitaceae, genus; *Momordica* and species; *charantia*. It has been documented to reduce fertility in both male and female animals (Kumar et.al, 2010).

*Momordica charantia* leaves were shade-dried and milled into fine particle sizes. Five isonitrogenous diets of 0g, 20g, 40g, 60g and 80g *Momordica charantia* leaf meal / kg diets were formulated to provide 35% crude protein using menhaden fish meal (65% crude protein), yellow maize, vegetable oil, vitamin-mineral premix, cod-liver oil and soybean. All the ingredients were milled, weighed and thoroughly mixed. 10ml hot water at 90°C was added at intervals to gelatinize the starch content and the paste formed was pelletized and air-dried at ambient temperature of 22°C for 72 hours to constant moisture content. Juvenile male and female *Oreochromis niloticus* (30.36±0.13g) were stocked in plastic tanks (1m x1m x1m) containing 400 litres clean borehole water. The experiment was replicated thrice. The fish were fed twice at 4% body weight/day for 80 days. Proximate analysis of the feed was carried out using AOAC, (1995) method, histological analysis of the gonads using Fitzsimmons (2005) method, Milt count using Sharma et.al. (2011) method and fecundity using Rana, (1985) method. Analysis of Variance (ANOVA) was used to test for significant differences in means and Turkey test ( $P>0.05$ ) for post-hoc test. Growth rate and feed conversion ratio were also determined.

Histological section of *O. niloticus* ovary fed 20g, 40g 60g and 80g MCLM/kg diet showed increase in interstitial cells, few oocytes in the ovary, abnormal gonadal development and necrosis while that of male testis showed increase in interstitial cells, disintegration in connective tissue and seminiferous lobule. As the concentration of *Momordica charantia* increases, fecundity and milt count also decreases (table 1). These results showed that *Momordica charantia* can be used to control the fertility of tilapia.

Table 1: Reproductive parameters from adult female and male *Oreochromis niloticus*

| Parameters                            | Concentration (g)   |                     |                      |                     |                      |
|---------------------------------------|---------------------|---------------------|----------------------|---------------------|----------------------|
|                                       | 0                   | 20                  | 40                   | 60                  | 80                   |
| Fecundity                             | 284.67 <sup>a</sup> | 179.00 <sup>b</sup> | 176.33 <sup>bc</sup> | 136.00 <sup>d</sup> | 112.00 <sup>ab</sup> |
| Milt count<br>x10 <sup>9</sup> (s/ml) | 3.59 <sup>a</sup>   | 2.21 <sup>b</sup>   | 1.67 <sup>c</sup>    | 1.18 <sup>d</sup>   | 0.27 <sup>e</sup>    |

Means with same letters are not significantly different using SPSS ( $P>0.05$ )

## EFFECTS OF INTENSE INBREEDING ON GROWTH TRAITS IN TILAPIA (*Oreochromis mossambicus*)

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Tilapia species plays an important food security role in developing countries, but is often farmed by farmers with very little technical knowledge about breeding. When animals are kept in groups and depend on natural mating, inbreeding is inevitable, as inducing spawning by artificial means is impossible. Large amounts of inbreeding in these farming communities may reduce production efficiency. In as much as inbreeding is unavoidable, understanding its effects may aid in the decision making processes of breeding programmes, especially in developing countries. The extent of inbreeding in small-scale production systems farming with tilapia should be estimated to create awareness and alleviate the effects of inbreeding. Therefore, the objective of study was to evaluate tilapia (*Oreochromis mossambicus*) productivity under the maximum possible inbreeding conditions. Full-sib matings were conducted on *O. mossambicus* for three generations at Welgevallen experimental farm at the University of Stellenbosch. A total of 25 males and 25 females were used as the parental stock, where a 14-day spawning period was followed by three generations of full-sib mating. Measurements of body weight (BW), standard length (SL) and specific growth rate (SGR) were recorded for each of the sixteen randomly sampled fish per replicate, at two weeks growth intervals for the period of 90 days at each generation. Regression analysis was used to determine the rate of phenotypic depression per unit increase in F and analysis of variance used to establish the difference between the means. The results show that BW, SL and SGR decreased with increase in inbreeding at each generation. Average inbreeding depression for BW ranged from 8.35 to 46.57%, while the average inbreeding depression per 10% increase of inbreeding ranged from -12.42 to -18.62%. For SL, the mean inbreeding depression was 18.15 and 17.95% at  $G_2$  and  $G_3$ , respectively. Inbreeding depression coefficients for SL were -7.2 % at  $F = 0.250$  and -4.79% at  $F = 0.375$  per 10% increase of inbreeding. The inbreeding depression for SGR was 21.76 and 20.34% at  $F = 0.250$  and  $F = 0.375$ , respectively. Per ten percent increase in inbreeding, inbreeding depression coefficient was -8.70% at  $F = 0.250$  and -5.43% at  $F = 0.375$  for SGR. These results show the extent of inbreeding that can be expected under extreme conditions where inbreeding is not controlled, especially where technical knowledge is lacking. They indicate that inbreeding reduces the performance of *O. mossambicus*; hence such decrease in performance may be addressed by minimizing inbreeding, which can be achieved by avoiding the mating of full-sibs. Therefore, if inbreeding is not checked in the small-scale production systems, overtime production will decrease



## **GENDER ROLES AND POST-HARVEST LOSSES ALONG AQUACULTURE VALUE CHAIN IN SOUTH WEST STATES OF NIGERIA**

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Catfish is the most (80%) cultured fish species in Nigeria which is consumed in various forms. However, post harvest losses are as high as 30-40%. These products can have narrow demand if not transformed into other intermediate forms. Value addition from production to processing and marketing result in better earnings by value chain actors, but there is inadequate information on patterns of value addition for most states in Nigeria. However, post-harvest losses usually are not included in the value chain analysis and gender roles in aquaculture are underestimated. Therefore, the study was aimed to estimate post-harvest losses, identify pattern of value chains and roles of women in aquaculture sector of Oyo and Lagos states.

Multistage sampling method was used to select actors in value chain of catfish in Oyo and Lagos States by proportionate sampling of Agricultural Development Programme (ADP) zones in each of the states. Producers, wholesalers, retailers and processors were sampled. Total number of respondents used for the study was 501 selected from membership list of Oyo State ADP, Lagos State Agricultural Development Authority Lagos State (LSADA), Lagos State Catfish Farmers Associations (LACAFA) and Catfish Farmers Association of Nigeria (CAFAN). Primary data were collected through in-depth interviews, focus group discussions and structured questionnaires. Post-harvest losses among the actors, relative proportion of gender and consumer preference were determined for each state. Statistical analyses used include descriptive statistics, t-test and multinomial logit regression analysis.

Socio-economic characteristics of actors along the chain reveal that women are more involved in processing, while men are into production and input supply. Post-harvest losses were recorded more by fish farmers. There was significant differences in post harvest losses recorded between both states ( $p < 0.05$ ). Information on pattern of post-harvest losses, value addition, roles of women in aquaculture would help strengthen policy recommendation about actors in aquaculture production. Policies that are not centered on gender will ultimately increase post-harvest losses and negatively impact livelihoods and food security on the long run.

## **ROLE OF COMMUNITY FOREST ASSOCIATION IN FOREST CONSERVATION: A CASE STUDY OF CHUKA BLOCK OF MOUNT KENYA FOREST, KENYA**

### **Sub-theme: Environment and Climate Change**

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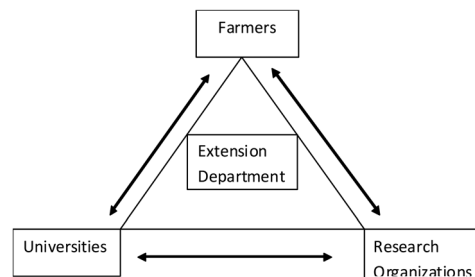
Forests are important resources providing ecological, social and economic benefits to millions of people in the world. In recent times, conservationists have underscored the need for creation of effective mechanisms for local communities' participation in conservation of these vital resources and the ecosystems they support. In Kenya, the introduction of Participatory Forest Management was realized upon the review of the Kenya's Forest Act in 2005 which led to formation of Community Forest Associations (CFAs). Recent studies have reported notable successes in CFAs' involvement in forests conservation. However, concerns have also been raised on the activities of CFAs since their roles have not been clearly defined, a situation that has led to conflicts with community members and other stakeholders like the Kenya Forest Service. In this study, we sought to determine the role of the CFAs in forest conservation, the benefits members obtain and the challenges they face. The study was conducted in Chuka forest block of the Mt Kenya Forest, in Tharaka Nithi County. Descriptive research design was used and data collected from CFA members, KFS & KWS officials using questionnaires, interviews and focused group discussions. Results showed that CFA members play vital roles in the forest conservation where they are engaged in activities like tree planting, educating the public on conservation, nursery establishment and management and reporting of illegal forest activities to authorities. We also established that the benefits they obtain from the forest were providing great impetus for the willingness of the members to participate in forest conservation activities. Although a number of challenges facing the CFAs were enumerated, there was a clear indication that their participation in conservation could be greatly enhanced by involvement of the KFS & KWS officials in their activities. We also established the need for both KFS and KWS to enhance and encourage these conditions so as avoid precipitation of any conflict of interests in the conservation of the Chuka Forest Block.

## ENHANCING GRADUATE TRAINING AND RESEARCH IN AQUACULTURE THROUGH INSTITUTIONAL COLLABORATIONS

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The ultimate aim of research at Higher Education Institutions (HEIs) of learning is societal problem solving. Sub-Saharan African aquaculture graduates have researched on several disciplines, including fish nutrition, production systems, fish health, breeding and processing. Unfortunately, many of their findings (even though interesting) are poorly disseminated and not sufficiently adopted by the beneficiaries, i.e., the farmers. Yet, graduate students usually investigate challenges that significantly affect society. There seems to be a disjointed linkage among African training institutions, research organizations, the farmers and the actual and potential extension workers (Fig.1). This does not only affect information flow, but also hinders effective training of graduate students, since very few of them would access the farms for hands-on training, yet most higher institutions in Sub-Saharan Africa are ill-equipped as far as practical and research facilities for training aquaculture graduates is concerned.



*Figure 1: An ideal extension model involving farmers, extension department, universities and research organizations*

We share the farmer centered approaches being adopted at Makerere University aimed at improving post-graduate training in fisheries and aquaculture. Like other Sub-Saharan Africa universities, Makerere offers specialized training in Aquaculture and Fisheries, but with limited facilities such as tanks, ponds, and cages. A modular training approach that utilizes facilities at research organizations and private farms is suggested. Besides, utilizing the scarce resources, this approach brings together the key stakeholders in the industry (i.e., the farmers, researchers, university lecturers and the students, who are the potential extension workers, Fig. 1) to work together. The farmers, researchers and tutors at higher institutions can freely collaborate in curriculum development and reviews, module development and training. This approach further reduces the cost of training, since the research institutions and farmers offer some requirements in kind; while the trainees offer practical solutions as the university lecturers offer technical backstopping. This approach stands to benefit all participants, and more so the graduate students who access facilities, hands-on training and multifaceted mentorship. To the university, competent graduates are produced at affordable cost, even though some additional operational costs have to be met. Further, the farmers are able to get timely and well researched solutions to their problems. We suggest and recommend this as a manageable approach to most Sub-Saharan African universities.

## ESTIMATION OF THE TOTAL, DIGESTIBLE AND AVAILABLE PHOSPHORUS REQUIREMENT IN PRACTICAL DIETS OF JUVENILE *Clarias gariepinus* BASED ON GROWTH, WHOLE BODY AND BONE MINERALIZATION

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Phosphorus (P) is an essential nutrient involved in many physiological processes in the body, including growth. However, level of P in aquatic environment is limiting for uptake by fish. Excessive use of inorganic phosphorus in fish diet as a supplement enriches aquatic environment and threatens the sustainability of aquaculture production. The P requirement has been determined for juvenile *Clarias gariepinus*. However, a quantification of the requirement on an available and digestible P basis is necessary to reduce P in farm effluents. The purpose of this study was to evaluate the phosphorus requirement of juvenile *Clarias gariepinus* on an available and digestible basis. Two consecutive feeding and digestibility trials were carried out to evaluate available and digestible P requirements of juvenile *Clarias gariepinus*. In trial 1, experiment 1, five (5) iso-nitrogenous ( $\sim 42.31\%$  crude protein) and iso-caloric ( $\sim 18.18\text{ MJ kg}^{-1}$ ) diets based on roasted, full fat soya bean with hulls were formulated to replace fish meal at 0 (S0), 25 (S1), 50 (S2), 75 (S3) and 100% (S4). The average total, available P, digestible P, and phytate P content of the diets (25-100%) were set at  $\sim 0.84\%$ ,  $0.34\%$ ,  $0.70\%$ , and  $0.48\%$ , respectively. Microbial phytase was supplemented at 0 (P0), 250 (P1), 500 (P2), 750 (P3) and 1000 FTU  $\text{g}^{-1}$  (P4) in a 5X5 factorial arrangement of ANOVA. Diet with 100% fish meal had no phytase supplementation. Fish ( $n=1638$ ,  $11.55 \pm 0.20\text{g}$ ) were randomly allotted to each tank, with triplicate group of 26 fish  $\text{tank}^{-1}$  and fed each diet at 3% body weight. Based on the result obtained in experiment 1, a diet with similar composition with 75% roasted soya bean was formulated and supplemented with 50, 100, 150, 200 FTU  $\text{g}^{-1}$  and inorganic P in a completely randomized design with three replications (experiment 2). Fish ( $n=390$ ,  $11.55 \pm 0.20\text{g}$ ) were hand-fed at 3% body weight. Both experiments were monitored for 84 days. Nutrient digestibility was measured using indirect method with 0.5% chromic oxide. Result in experiment 1 (trial 1) showed that there was a significant reduction ( $P < 0.05$ ) in WG, SGR, PER, and an increase in DFI, FCR, and survival rate with increasing substitution of fish meal. There was significant interaction between soya bean and phytase on WG, SGR, PER, FCR, and DFI ( $P < 0.05$ ). Phytase supplementation improved growth significantly ( $P < 0.05$ ). Fish fed P1 recorded the highest WG, compared to P0, P2, P3 and P4 ( $P < 0.05$ ). SGR was highest in P1, but was not significantly different from P0, P2, P3 and P4 ( $P > 0.05$ ). The effect of phytase on HSI, CF, and survival rate was insignificant ( $P > 0.05$ ). Protein digestibility was highest in (P4). P retention increased significantly ( $P < 0.05$ ), being highest in P1. Faecal P increased with phytase supplementation ( $P < 0.05$ ). The highest and least bone P and calcium were obtained in P1 and P4, respectively. In experiment 2, the result showed that WG, SGR, PER were significantly lower ( $P < 0.05$ ) in 50, 100, 150, 200 FTU  $\text{g}^{-1}$ , and inorganic P compared to 250 FTU  $\text{g}^{-1}$ . The Fish fed 50, 100, 150 and 200 FTU  $\text{g}^{-1}$  had significantly lower CF compared to 250 FTU  $\text{g}^{-1}$  ( $P < 0.05$ ). The ADC for protein, lipid and body protein were higher in 250 FTU  $\text{g}^{-1}$ , compared to 50, 100, 150, 200 FTU  $\text{g}^{-1}$  and inorganic P ( $P < 0.05$ ). However, fish fed 50-200 FTU  $\text{g}^{-1}$  had higher ADC for P, serum P, body P, ash, moisture, bone P, and lower faecal P compared to 250 FTU  $\text{g}^{-1}$  ( $P < 0.05$ ). In trial 2, iso-nitrogenous ( $\sim 42.18\%$  crude protein) and iso-caloric ( $\sim 17.71\text{ MJ kg}^{-1}$ ) diets in which fish meal was replaced by increasing levels of roasted and oil-pressed groundnut meal (dehulled) at 10% (G1), 20% (G2), 30% (G3), 40% (G4), 50% (G5) and 60% (G6) were formulated. The average total, available, digestible and phytate P were  $\sim 0.88$ ,  $0.47$ ,  $0.75$ , and  $0.42\%$ , respectively. Phytase was supplemented at P0, P1, P2, P3 and P4. Fish ( $n=2340$ ,  $14.56 \pm 0.20\text{g}$ ) were randomly allotted to triplicate tanks (26 fish  $\text{tank}^{-1}$ ) and fed at 3% body weight for 84 days. Phytase supplementation improved WG, FCR, SGR, and PER significantly compared with control diet ( $P < 0.05$ ). HSI and CF were significantly reduced in all phytase diets compared to control ( $P < 0.05$ ). The increase in ADC of P was insignificant at all level of phytase compared to control ( $P > 0.05$ ). A significant increase in serum P ( $P < 0.05$ ) and a reduction in faecal P ( $P > 0.05$ ) was recorded in phytase-supplemented diets compared to control. Bone P declined significantly as the phytase dose increased, with the least bone P and highest iron in P4 ( $P < 0.05$ ). Bone calcium and zinc were significantly least in P1 and highest in P4 ( $P < 0.05$ ). In conclusion, the research revealed that a low P ingredient increases the P requirements for juvenile *Clarias gariepinus*, with a total P ranging from 1.076 to 1.266%. Based on growth, bone P and whole body P, the available P requirements were 0.75-0.80%, 0.87-1.01%, and 0.51-0.65%, respectively. The digestible P requirements were 0.79-0.94% (SGR) and 0.68-0.84% (whole body P).

## **EFFECT OF COMMUNITY DEVELOPMENT OUTCOMES ON SEAFOOD ACCESSIBILITY AND CONSUMPTION IN TANZANIA**

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Unlike most African countries, Tanzania is able to produce 90 percent of its food even though it is amongst the poorest countries in the world. However, high post-harvest losses, incompetent distribution systems, low income and the dependence on rainfall agriculture contributes to a high food insecurity problem. The agricultural sector employs about 70 percent of the labor force and in 2003; agriculture contributed about 45 percent to the national GDP. The fisheries sector is also a growing industry, and it contributes to the economy through employment, income generation, foreign earnings and revenue for the country. It is a source of livelihood for about 4 million people. Fish consumption provides about 30 percent of animal protein for households but per capita fish consumption in 2013 was 7.7kg, which is below the global average of 19kg. The government of Tanzania is trying to increase accessibility to food through price reduction on staple foods to assist poor households during low production periods. Consumption is also being encouraged through increased production in food insecure regions. This implies an improvement in the connection between food insecure regions to market hubs. However, a recent World Bank study identified that improving agricultural productivity does not automatically increase accessibility to food.

The most productive agriculture and fisheries regions in Tanzania are the in Southern Highlands (Rukwa, Mbeya, and Iringa) and Kagera, Mwanza and Mara in the Lake zone. These areas face high transportation cost to transfer produce to regions in and around Dar es Salaam, Shinyanga and Dodoma. Areas in the Southern Highlands have the lowest calorie share from fish in the country. Poor road systems, inefficient market distribution systems and infrastructure such as post-harvest storage systems beset accessibility to agricultural produce including seafood in many developing countries. We hypothesize that roads (proxy for access to markets), electricity (proxy for supply in terms of storage) and mobile networks (proxy for marketing) are significant determinants of seafood accessibility in Tanzania.

The main objective is to identify how community development outcomes influence the accessibility and consumption of households to seafood in Tanzania. We use the food basket approach with fish calorie shares to measure accessibility and the Food Consumption Score (FCS) from the World Food Program to measure consumption. We use the FCS since it weights each food item differently. Accessibility and consumption will be analyzed as functions of household demographics, community infrastructure, government policies and prices of other complementary and substitute animal protein sources in a Seemingly Unrelated Regression (SUR) framework. Data source is the 2011/12 Tanzania Household Budget Survey.



## AQUACULTURE AND FOOD SECURITY: AN ASSESSMENT OF FISH FARMING HOUSEHOLDS IN GHANA

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Fish farming has become an important part of the Ghanaian economic development plan for the past ten years. Its importance was further enhanced with the setting up of the Ministry of Fisheries and Aquaculture Development in 2013 to give more emphasis and support to the industry. Several international organizations like the World Bank, Food and Agriculture Organization (FAO), UK's Department for International Development (DFID), the New Partnership for Africa's Development (NEPAD) and local ones like Ghana Association of Women Entrepreneurs (GAWE) and Rural Wealth (RW) have all contributed to the development of this industry through financial support and capacity building.

In spite of these efforts and contributions, there is little assessment on the influence of participating in fish farming on the nutritional quality of such households. We hypothesize that fish farming households have higher food consumption scores than non-fish farming households. The assumption is that engaging in fish farming will increase income flow and access to fish for the household. Thus, the main objective of this study was to identify the direction of impact of fish farming on household food security and the pathways of impact. We adopted the Propensity Score Matching (PSM) approach in a logit framework to evaluate participation in fish farming. The dependent variable was World Food Program's (WFP) Food Consumption Score (FCS), a proxy for food security. Socio-economic variables including *wealth index*, *ecological zone* and demographic characteristics of the household head including *Age*, *education in years*, *peri-urban*, *marital status*, *employment status*, *sex*, *household income per capita*, and *household size* were used as regressors. Our data sources are the 2013 Ghana Living Standard Survey (GLSS) and field data collected from in Ashanti and Brong Ahafo regions in June/July 2014.

Results showed that the average FCS for fish farming households was 69 while that of non-fish farming household (control group) was 57. On the margin, the probability of adopting fish farming increased with wealth index, residents of peri-urban area, ecological zone and household size but decreased with household income per capita. The average treatment effect on the average fish farming household (ATT) showed an increase in food security score by 14 points which translates into consuming fish at least twice in a week (=8), roots and tuber or cereals (=2), pulses and legumes once (=3) and vegetables or fruits (=1) once in a week. We infer that fish farming increases the diversity and frequency of food consumed through direct consumption and not so much through the income effect. Post-estimation analysis showed that households in the savannah zone with an opportunity to engage in fish farming especially in the rural areas have a higher probability of improving their food security status.

## **A LATENT-CLASS ANALYSIS OF HOUSEHOLD DEMAND FOR SEAFOOD IN GHANA**

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Fish is an integral part of Ghanaian diets and it contributes over 60 percent of the animal protein for human consumption. It is the cheapest source of animal protein with the average Ghanaian consuming more fish than meat products. The per capita consumption of fish is about 25kg per annum, which is one of the highest in Sub-Saharan Africa. It is commonly consumed by low income and subsistence households. A survey conducted between 1987 and 1999 showed that fish accounted for 13 to 19 percent of urban household average food budget and 17 to 29 percent for rural households. In 1998/1999, the expenditure on fish as a proportion of expenditure on animal protein was 53 percent for urban households and 55 percent to 79 percent for rural households. The fifth round of the Ghana Living Standards Survey showed that fish accounted for 27 percent of the overall household food budget. According to European Commission(EU), consumption patterns in developing countries are beginning to follow the pattern in developed countries which is mainly, decreased daily consumption of animal protein particularly meat but increased fish consumption.

The literature suggests that changes in income per capita and health-related factors have been driving animal protein consumption in both developed and developing countries. Other drivers include ethical factors, environmental and economic issues, availability and urbanization, as well as socio-economic and demographic factors. This study therefore examines the effect of price and income, socio-demographic factors, as well as cultural, health and lifestyle factors on household demand decisions for seafood. This study will provide insights into lifestyle factors that influence the demand decision of households and aid producers in the marketing process in targeting consumers in certain demographic groups.

Data from the 2013 round six of the Ghana Living Standards Survey (GLSS 6) will be used to assess the determinants of seafood demand. The proposed methodology is the Latent Class Linear Regression Approach to help capture the heterogeneity among the households and any unobserved factors underlying the demand decision process.

## A GENETIC STRUCTURE STUDY OF THE SCALLOPED SPINY LOBSTER *Panulirus homarus* FROM OMAN

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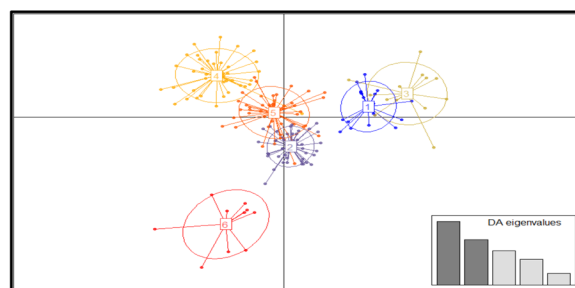
The scalloped spiny lobster (*Panulirus homarus*) is an important aquaculture species worldwide, and a major export commodity for many countries in the Indo Pacific Zone (IPZ). In Oman, a sharp decline in landings (2000 tons in the 1980s to only about 416 tons in 2015) of this species has been reported over the last three decades. Hence, concerns about the sustainability of the current fishery management approach have been raised. For the successful fishery management of the scalloped spiny lobster, it is vital to understand the population genetic structure and to delineate the boundaries of unique genetic stocks. This study demonstrates the practical application of a seascape approach, utilizing genome-wide single nucleotide polymorphism (SNP) markers, and provides novel insights into the genetic structure of *P. homarus* populations across the Omani coast.

To assess population structuring and genetic differentiation of *P. homarus* populations across the Omani coastline, a total of 180 pleopod samples were collected from nine sites (Fig. 1). A reduced representation sequencing GBS approach was used for SNP discovery (DArTseq - Diversity Array Technology Pty Ltd), and a total of 3095 highly informative markers were selected for analysis following stringent filtering. In addition, ecological data such as temperature, salinity, seafloor topology and hydrodynamic dispersal modelling were used to address potential environmental effects on genetic structure of *P. homarus*.

Six clear genetic clusters of *P. homarus* appear to be present within Omani waters (Fig. 2). These findings suggest that fishery managers should consider these stock boundaries as distinct management units. Additionally, findings here highlight the potential of seascape features to impose local population sub-structuring of the scalloped spiny lobster across Omani coast through differential selective pressures.



**Fig. 1** Sampling sites of *P. homarus* specimens collected from Oman coast.



**Fig. 2** Discriminant Analysis of Principal Components (DAPC) scatter plot using 180 *P. homarus* individuals, in the R package adegenet. Dots represent individuals.

NOVEL METABOLIC AND IMMUNOLOGICAL BIOMARKERS IN OYSTERS CHALLENGED WITH A VIRULENT STRAIN OF OSHV-1

Andrea C. Alfaro\*, Tim Young

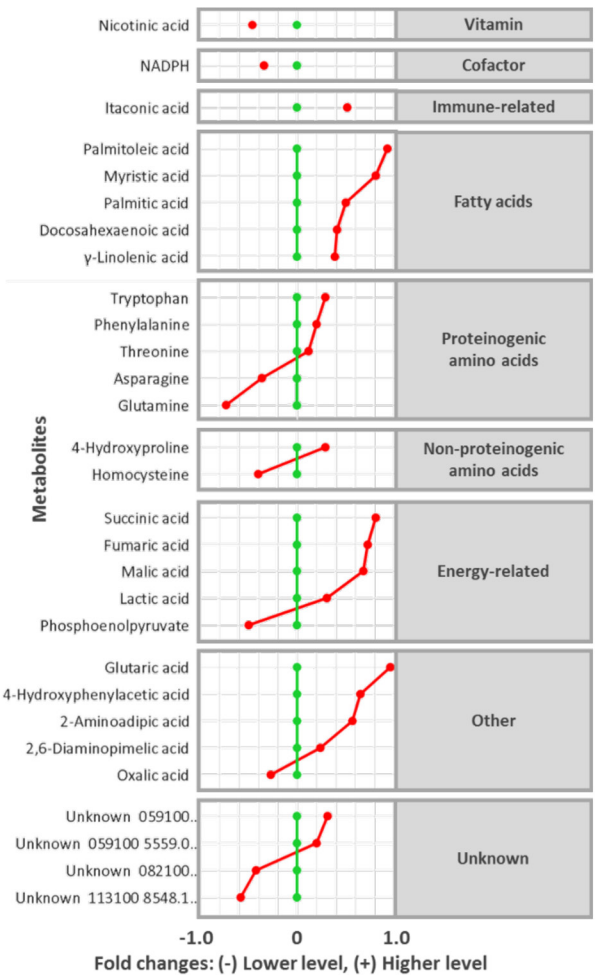
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A novel metabolomics approach was used to identify metabolic and immunological responses in oyster larvae exposed to OsHV-1  $\mu$ Var. Multivariate analyses of entire metabolite profiles were used to separate infected from non-infected larvae. Correlation analysis revealed the presence of major perturbations in the underlying biochemical networks and secondary pathway analysis of functionally-related metabolites in virus-exposed larvae. These results provide new information about the pathogenic mechanisms of OsHV-1 infection and susceptibility in oyster larval hosts. In addition, it is expected that this work will assist with disease mitigation strategies and/or as new phenotypic information for selective breeding programmes aiming to enhance viral resistance.

We performed a comprehensive determination of metabolic alterations in oyster larvae exposed to the newly emerging and highly virulent OsHV-1  $\mu$ Var genotype via GC/MS-based metabolomics. Our findings revealed that viral exposure had an effect on many metabolites involved in central carbon metabolism, across broad chemical classes with various functional roles. These virus-induced changes in the metabolite profiles enabled us to discriminate healthy from unhealthy larvae via multivariate clustering and classification techniques, discern relationships among metabolites, identify entire biochemical pathways evidenced of being altered, and further focused our attention towards specific mechanisms of immunity characteristic of the pathophysiological condition. We identified coordinated changes in tricarboxylic acid (TCA) cycle-related metabolites in virus-exposed larvae indicative of abnormal energy metabolism and biosynthesis of an antimicrobial product, and also detected subtle signs of potential oxidative stress, transformation or degradation of extracellular matrix scaffolding, and disruption of normal lipid metabolism suggestive of requirements for viral appropriation of host-cell biomaterial, among other processes.

Our study also highlights the value of metabolomics-based approaches in elucidating host-virus interactions and the metabolic networks which characterise and underpin the pathophysiological state, and further supports its application for investigating pathogenesis of disease in early life stage molluscan models.

**Figure 1.** Summary of statistically different metabolite levels between treatment groups with their respective Log<sub>2</sub> fold change values (virus-infected [red circles] / control [green circles] larvae).



## SWITCHING FROM PLANNING TO IMPLEMENTATION, THE SEYCHELLES TALE TO THE BUILDING OF A NEW AQUACULTURE INDUSTRY

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The Seychelles Island is made up of 115 islands which occupies an Economic Exclusive Zone (EEZ) of 1.3 million km<sup>2</sup>. Seychelles economy is mostly based on tourism and fisheries due to its pristine environment and large EEZ. In 2008 however with the global economic crisis, Seychelles had to explore the possibility of diversifying its economy. Aquaculture was thus identified as a new potential industry. Since then various specialist studies has been conducted which led to the development of a 'Mariculture Master Plan' (MMP).

The MMP functions as the backbone unto which the industry will be developed. The MMP includes regulatory frameworks, baseline studies, economic plans, and best management practices in Aquaculture from some of the world leaders in the field.

The government through the Seychelles Fishing Authority (SFA) will develop and manage three pilot projects leading to the launch of the new industry; 1. A Broodstock, Acclimation and Quarantine Facility (BAQF); 2. A grow-out pilot sea cage facility; 3. A Research and Development (R&D) facility. The MMP states that the first two kilometres from shore will be reserved for small local investors, most of which would not have the funds to build their own BAQF among other factors. The pilot grow-out sea cages will be built as a case study for potential investors, as they would want to know if aquaculture is viable and economical in the Seychelles. The R & D facility will be located in the campus of the University of Seychelles and is anticipated to become the Centre of Excellence (CoE) in the Western Indian Ocean Region (WIOR) on matters of aquaculture.

Seven indigenous marine species have been selected to kick start the industry, those were selected following strict selection criteria (fig 1).various other species have been highlighted for potential R & D studies as the industry progresses.

An Environment and Social Impact Assessment (ESIA)which was done over a 9 month period by Golder Associates (EIA experts) and was approved by the Ministry of environment (MEECC) in February 2017. The ESIA report provided various mitigating measures and re-assured all parties that should these be followed, the aquaculture industry would have more positive outcomes and benefits than negative ones for the Seychelles.

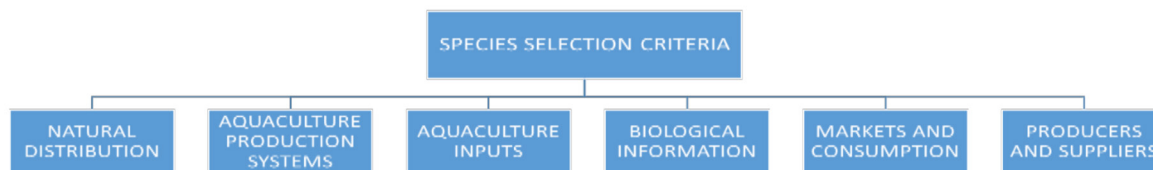


Fig 1.



## CHALLENGES AND PROSPECTS FOR SEA CUCUMBER POND CULTURE IN THE PHILIPPINES: THE CASE OF SANDFISH *Holothuria scabra*

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Development of culture ponds in the Philippines has been rapid in the 1980s when there was a boom in tiger shrimp *Penaeus monodon* production. However, the spread of shrimp diseases since the 1990s has rendered many of these ponds abandoned, while some shifted to culture milkfish *Chanos chanos*. This paper seeks to explore the potential of rearing sea cucumbers, particularly the sandfish *Holothuria scabra*, in these typical ponds in the Philippines, since it was shown that pond culture of sandfish in shrimp ponds was successful in Viet Nam in the past decade. The Western Visayas region in the central Philippines is where most culture ponds were constructed by typically converting intertidal mangrove areas. Field surveys were conducted among representative ponds in this region to assess their status and suitability for sandfish culture using presently known parameters (e.g. salinity, sediment type, etc.). Culture experiments were conducted to evaluate growth and survival of sandfish under different culture space, sediment quality, seasonality, and water management regimes.

Initial growth spurt was consistently recorded during the first 2 weeks of culture where sandfish juveniles (3-5 g) can grow by 1.3 g d<sup>-1</sup>. Regardless of initial stocking size and culture space/area, sandfish more than doubled their size in less than 1 mo without supplement feeds. The typical silty-muddy sediments of ponds were less productive (low growth) and often toxic (total mortality) to sandfish; while more sandy sediments produced bigger sandfish with higher survival (>80%). Practical water management with natural tides to partially drain then replenish pond water worked adequately during fair weather (1-3 mo). However, slow growth and/or high mortality were experienced during unfavorable weather conditions. Prolonged rains during the wet season (June–October) result to stratification of the water column with decreased salinity and temperature. On the other hand, prolonged heat during dry months (November–May), especially during El Nino event, result in intense water temperature of the shallow (<1m) ponds. This unique monsoonal seasonality limits culture duration of sandfish.

Generally, typical ponds in the Philippines are not ideal for sandfish culture, mainly because of: (1) tendencies of low salinity, being typically situated in brackishwater area; (2) substrate being too muddy and silty, having been converted from mangrove areas and were intensively used for shrimp or milkfish; (3) being typically shallow at <1 m; and (4) climate and weather that limits culture duration. However, even with these inherent limitations, using the ponds as a short-duration (1-3 mo) nursery system for sandfish juveniles showed good prospects with results revealing fast growth and high survival. To further increase prospects for growing sandfish to marketable sizes in Philippine ponds, substantial modifications may be needed (e.g. increasing depth, re-conditioning of sediments, installation of aeration and water exchange systems, and provision of supplemental feeds), but with considerably high financial requirements.

## UNDERSTANDING BIO-PHYSICAL VARIABILITY IN SEA CUCUMBER RANCHING SITES IN THE PHILIPPINES

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A number of sea cucumber sea ranch are being established in the Philippines, to enhance stocks of sandfish *Holothuria scabra*, while providing a supplemental livelihood to local communities and improve environmental protection of the site. Characterization of the sea ranch area is important to establish the biophysical parameters to identify good sea ranching sites. In this study, four ranch sites in different regions were assessed, located at: (1) Victory, Bolinao, Pangasinan [VIC], (2) Polopina, Concepcion, Iloilo [POL], (3) Maliwaliw, Salcedo, Eastern Samar [MAL], and (4) Tubajon, Laguindingan, Misamis Oriental [TUB]. A 7x7 grid (49 sampling points) was imposed on each 5Ha ranching site. At these points, biophysical parameters including seagrass cover (%), penetrability (cm), organic matter (%OM), Chlorophyll-a concentration (ug/g), and sediment particle size composition (%) were assessed using standardised protocols.

Principal Components Analysis (Fig. 1) revealed that fine sediment particles (<125  $\mu$ m) are positively correlated to OM content, while coarse sediment particles (>500  $\mu$ m) correlate with chlorophyll-a and phaeopigment concentrations. Medium-sized sediment particles (125-500  $\mu$ m) correlate with seagrass cover. VIC is characterized mostly by coarse sediment particles and relatively higher chl-a loading. In contrast, POL has mostly fine particles with inherently higher %OM than other sites. Meanwhile, TUB is characterized by higher seagrass cover and, to a degree like MAL, mostly composed of medium sediment particles.

Past evidence from ranching trials shows that a site that exhibits good survival and growth in a given year may not necessarily retain these qualities between years. Our high-resolution monitoring across seasons and years shows considerable dynamism in sediment bio-physical properties.

Among these sites, to-date only VIC has shown good signs of growth and harvest of sandfish, providing tentative evidence that sea ranching sites with coarser sediments, high chl-a levels, but low OM and seagrass cover, may be optimal.

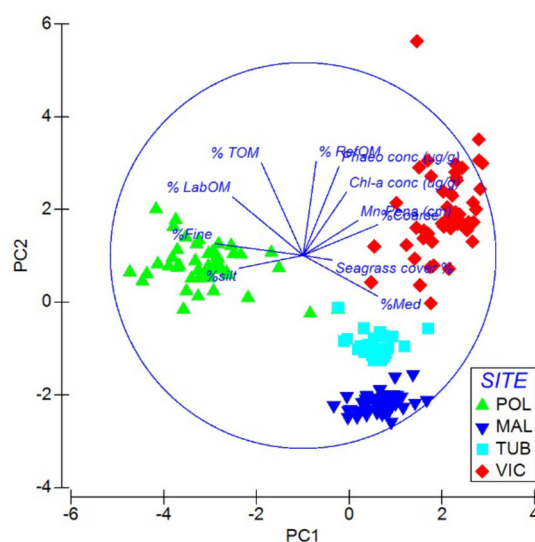


Fig. 1. PCA of biophysical parameters associations and characterization of 4 sea ranch sites in the Philippines.

## STOCK ENHANCEMENT OF SHRIMPS IN THE PHILIPPINES AND ITS IMPACT ON FISHERIES AND THE COMMUNITY

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The New Washington Estuary (NWE) in central Philippines was a productive shrimp fishing ground in the 1970s, where a fisher can haul up to 24 kg of fresh catch daily. The highly-priced tiger shrimp *Penaeus monodon* composed majority of catch by weight during those times. However, harvest volume declined by almost half every decade, bringing down per capita catch to only 0.7 kg in 2013. This decline was coincidental with the conversion of 92% of natural mangroves into culture ponds and >400% increase in fishing gears after 1980s. A project by the Research Institute for Humanity and Nature (RIHN) of Kyoto, Japan, in collaboration with The Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) in the Philippines, conducted a Community-Based Shrimp Stock Enhancement Sub-Project in the NWE to evaluate prospects of restoring shrimp stocks and increase income of local poor fishers. From 2013 to 2017, seven culture-and-release runs have been conducted with successful releases of a total of over 650,000 shrimps.

Before the stock enhancement activities in 2013, baseline monitoring showed a low chance of catching tiger shrimps at a rate of only one piece out of 25 harvests. After some releases were conducted, tiger shrimp catch increased by 250% in 2014 and 440% in 2015. Tag and capture studies also indicated a conservative recovery rate of 8%. However, monthly monitoring indicated an unsustained increase in catch only immediately after the releases. Then a corresponding steep decline in harvest was evident in succeeding months, indicating intense and unregulated fishing pressure. In the social aspect, the local community expected good benefits from stock enhancement. 92% believed to achieve improved income from catch, 81% expected additional food for family, 68% thought it can revive the estuary, and 63% expected better community solidarity. During the course of the project, people perceived to have a generally improved living condition brought about by increase in shrimp catch. However, their interest and willingness to actively participate in stock enhancement activities declined from 77% in 2013 to 56% in 2015. This can be attributed to a perceived unequal sharing of benefits by various community members, as well as their low rating for law enforcement and government management.

Shrimp stock enhancement activities in NWE demonstrated the feasibility of increasing catch and livelihood. However, more challenges must be overcome especially in the social and governance aspects. Education campaigns are needed to increase cooperation and involvement by community and government for more efficient law enforcement and management in order to achieve a more equitable and sustainable benefits from stock enhancement.

## IMPORTANT EXOTIC ORNAMENTAL FISH SPECIES IN NIGERIA

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A total of 300 aquaria in homes, offices and public buildings, ornamental fish importers' facilities, aquarium shops and ornamental fish breeding facilities in Nigeria were surveyed between January and August 2016 to identify the different fish species present.

A total of 45 exotic fish species were identified from 2,950 fish analyzed. Varieties of *Carassius auratus* (goldfish); *Pterophyllum Scalare*; (Angel fish) and *Tricogaster leeri* (Gourami) were the most common and found in 65%, 60% and 53% respectively of the tanks surveyed. While *Scleropages formosus* (Golden Arowana) and *Symphysodon aequifasciatus* (Discus fish) were the rarest species. 22.2% of the identified species are being bred locally. Only one of the fish tanks and facilities surveyed housed marine ornamental fish.

## POPULAR EXOTIC ORNAMENTAL FISH SPECIES IN NIGERIA

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## **KENYA WOMEN IN FISH FARMING AND MARKETING - MODERN PERSPECTIVES**

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Globally, across cultural, social, political, and economic strata, the role of women in aquaculture is more significant than commonly reported. Aquaculture in Africa has been promoted as a development strategy as it enables poor women to operate low technology and low input systems that are an extension of their domestic tasks. Entry into aquaculture appears to have fewer gender barriers, perhaps because this sector developed outside cultural traditional gender roles. Women have assumed a leading role in the rapid growth of aquaculture that includes farming, processing and marketing (fish, shrimps, sea-weed, and crab). Women participation along the aquaculture value chain is higher than in capture fisheries. In 2009 through 2014, the Kenyan government in seeking to alleviate poverty amongst the poor and ease fishing effort on wild fish stocks targeted Kenyan youth and women who constitute a large proportion of the rural poor for support in establishing aquaculture as an income generating activity through the Economic Stimulus program. The principle behind this approach was that improving women's income, access to information, technology, and decision-making processes would have a profound effect on the quality of life at both household and community levels. At the end of the program, a total of 48,000 fishponds had been constructed. Of these, women owned twenty two percent (22%) of those ponds. The program adopted a cluster approach in developing aquaculture, thereby providing women with numerous points of entry along the value chain, from production, input supply, processing, to marketing and retail. Currently, aquaculture's contribution to the national economy averages KES 9.4Billion, and that includes the contribution from the 22% of the ponds constructed under the Economic Stimulus program that are strictly owned by women / women groups. This percentage is on the increase due to technological advances along the aquaculture value chain and also the many programmes concerned with economic advancement/empowerment of women.

## POTENCIAL OF CLIMBING PERCH, *Anabas testudineus* CULTURE AT BRACKISH WATER AREA

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A study on the effect of water salinity ranging from 0-30 ppt on hatching success of climbing perch, *Anabas testudineus* was conducted in a 15-liter glass aquarium (water volume 10 liters) containing 500 eggs for various level of water salinity. Fertilization rate at 0, 5, 10, 15, 20, 25 and 30 ppt were 76.67, 61.33, 77.00, 47.33, 0, 0 and 0 %, respectively. The fertilization rate at 0-10 ppt water salinity was significantly ( $P<0.05$ ) higher than that at 15, 20, 25 and 30 ppt water salinity. Hatching rate at 0, 5, 10 and 15 ppt were 91.33, 87.90, 86.20 and 77.49 %, respectively. The hatching rate at 0-10 ppt water salinity was significantly ( $P<0.05$ ) higher than that at 15 ppt water salinity. The times of hatching out at water salinity of 0-15 ppt were between 1,255-1,300 minutes.

The survival tolerance of climbing perch larvae in different water salinity (0-30 ppt) within 24 hour were then studied using a 50-liter glass aquarium (water volume 30 liters) containing 50 larvae at each level of water salinity. All treatments were done in triplicate. The survival rate of fish larvae in the 0, 5, 10, 11, 12, 13, 14, 15, 20, 25 and 30 ppt water salinity were 100, 100, 100, 83.3, 83.3, 83.3, 70, 70, 0, 0 and 0 %, respectively. The survival rate at 0-12 ppt water salinity was significantly ( $P<0.05$ ). The gain rate of total body length at 8 weeks at water salinity ranging from 0, 5, 10 and 12 ppt were 1.33, 1.41, 1.36, 1.43 and 1.63 cm, respectively. The gain rate of total body length at 0-12 ppt water salinity was not significantly ( $P>0.05$ ). Water salinity in the range of 0-12 ppt was suitable for climbing perch culture.



## EFFECTS OF WATER SALINITY ON HATCHING OF EGG, GROWTH AND SURVIVAL OF LARVAE AND FINGERLING OF SNAKE HEAD FISH, *Channa striatus*

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A study on the effect of water salinity ranging from 0-30 ppt on hatching success of snake head fish, *Channa striatus* were conducted in a 15-liter glass aquarium (water volume 10 liters) containing 500 eggs for various level of water salinity. Fertilization rate at 0, 5, 10, 11, 12, 13 and 14 ppt were 69.33, 72.67, 71.33, 72.67, 82.00, 73.33 and 10.67%, respectively. The fertilization rate at 12-13 ppt salinity was significantly ( $P<0.05$ ) higher than 0, 5, 10, 11 ppt salinity. Hatching rate at 0, 5, 10, 11, 12, 13 and 14 ppt salinity were 60.00, 60.67, 66.67, 72.00, 77.33, 68.00 and 2.67%, respectively. Hatching rate at 12 ppt salinity was significantly ( $P<0.05$ ) higher than 0, 5, 10, 11, 13 ppt salinity. For the snake head fish, the water salinity of 12 ppt was shown to give the highest fertilization rate and hatching rate. The time of hatching out at water salinity of 0-14 ppt were between 1,192-1,442 minutes.

After hatching, the survival tolerance of snake head fish larvae in different salinity (0-30 ppt) within 24 hour were then studied using a 50-liter glass aquarium (water volume 30 liters) containing 50 larvae for each level of salinity. All work were done in triplicate. The survival rate of fish larvae in the 0, 5, 10, 15, 16, 17, 18, 19, 20, 25 and 30 ppt salinity were 100, 100, 100, 100, 0, 0, 0, 0, 0, 0 and 0 %, respectively. However, fish larvae that had survived at 15 ppt died after 8 days of rearing. The gain rate of total body length, body weight gain rate, and the survival rate (%), the experiment was done in 10 weeks at water salinity ranging from 0, 5 and 10 ppt was significantly ( $P<0.05$ ) higher than 11, 12, 13 and 14 ppt salinity. However, there were no significance differences ( $P>0.05$ ) among 0, 5, 10 ppt.

## COMPARATIVE STUDY ON THE HATCHABILITY AND SURVIVAL RATES OF CROSSES BETWEEN WILD AND FARMED CATFISH *Clarias gariepinus* Burchell, 1822 LARVAL PRODUCTION USING CATFISH PITUITARY EXTRACT HORMONE

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The African sharptooth catfish, *Clarias gariepinus* is one of the most sought after fish having an almost Pan-African distribution. Commercial breeding is achieved using hormones, including the catfish's pituitary extract, which is also used in this present study. This study aimed to ascertain whether there are remarkable differences in the fecundity, hatchability and survival rates between crosses of wild and farmed *Clarias gariepinus*; explicitly indicating the crossing with high quality seeds in sufficient quantity. Twenty-four broodstock selected weighed between 0.7kg-0.9kg for cultured and 0.45kg-0.8kg for wild catfish. The samples were put into four treatments; A(Farm Female, FF – Farm Male, FM), the control; B(FF- Wild Male, WM), C(Wild Female, WF-FM), and D(WF – Wild Male, WM), with three replication each. Males were sacrificed to remove their pituitary gland and testes (preserved 4°C in fridge). Females under each treatment were injected with 2ml pituitary gland solution. Mean weight of stripped eggs after 14 hours latency period for treatment A, B, C and D were 39.5g (12,719 eggs), 37.2g (11,978 eggs), 32.4g (9,882 eggs), and 30.6g (9,333 eggs).

Hatching was observed 24 hours after incubation, lasting for 33 hours at 23°C temperature. Percentage hatchability for treatments A (75%), B (68%), C (57.5%), and D (40%) were noted. There were significant differences  $p < 0.05$  between treatment A, and the rest of the treatments. Percentage survivals for treatments A(65%), B(53%), C(48.9%), and D(23.6%) after the 14th day of study were known. There were significant differences  $p < 0.05$  between the means of treatment A, treatment B, treatment C and D but there were no significant difference  $p > 0.05$  between treatment B(FF–WM) and treatment C(WF–WM). Treatment A with crossing of farmed female to farmed male broodstock gave the highest fecundity, hatchability and survived fries; hence should be used during the induced breeding of catfish. Farmers should always maintain some breeds in their farms as broodstock for subsequent fingerling production.

## THE EFFECTS OF VARYING BROODSTOCK PAIRING RATIO IN INDUCED SPAWNING ON THE HATCHABILITY OF CATFISH *Clarias gariepinus*, Burchell, 1822 EGGS

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African sharptooth catfish, *Clarias gariepinus* is one of the most farmed fish species in West Africa, where commercial breeding is done using hormones including pituitary gland. This study was carry out using natural pituitary from male catfish for induced breeding of *C. gariepinus* under varying brood stock ratios. The broodstocks selected weighed between 800g and 1000g males, 900g and 1000g females. They were used for three induced breeding trials in treatment ratios of A (1 Male, M:1 Female, F), B (2M:1F) and C (1M:2F). Females under treatments A and B were injected with 2mls of the pituitary solution each, and C (1M:2F) was injected with 1 pituitary (0.5 each ) of 1ml each. Fecundity of females in A was 10500 eggs, B was 12600 and the two females of C were 9600 and 10200 eggs (added up to get 19800 eggs). The weight of eggs produced were A (1F, 35g), B (1F, 42g) and C (2F was 32 and 33g, mean= 33g).

Hatching started 24 hours after incubation and lasted for 33 hours. Percentage hatching for treatment A was 51%, B was 75% and C was 23%. The differences between the percentages showed that there was no significant difference  $p > 0.05$  between A(1M:1F) and B (2M:1F). Treatment C (1M:2F) showed no significant difference ( $p > 0.05$ ) as compared to treatment A(1M:1F). The percentage survival of the treatments indicated that C(1M:2F) was the highest 49.0% followed by A (1M:1F), 30% and B(2M:1F), 26%. There was no significant difference  $p > 0.05$  between A(1M:1F) and C(1M:2F) but treatment A(1M:1F) and B(2M:1F) showed significant difference ( $p < 0.05$ ). Treatment B with two males and one female broodstock had the highest number of stripped and fertilized eggs, hatchlings, low number of spoiled eggs and high survival rate. Fish farmers should use the broodstock ratio of 2 males to 1 female for induced spawning to help them get enough good seed for their fingerling production, and also use larger receptacles for higher hatchability.

## NILE TILAPIA *Oreochromis niloticus* POPULATION GENETIC STUDIES WITHIN THE VOLTA RIVER BASIN: APPLICATION IN AQUACULTURE DEVELOPMENT IN GHANA

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Nile tilapia, *Oreochromis niloticus*, is the primary species contributing to aquaculture expansion in Sub-Saharan Africa within the last decade. However, little is known about the genetic variability within the species in the region and the performance of regional strains under current and future climate conditions in specific countries. Knowledge of the genetic variability within the species is vital for advancing aquaculture in Africa, through the selection of appropriate resource stocks.

The objectives of this study were to determine the genetic diversity and differentiation of wild populations of *O. niloticus* from the Volta River basin, and to characterize the phenotypic variation for tolerance of high temperature, low dissolved oxygen and high salinity. We collected *O. niloticus* samples for genetic analysis from three rivers within the Volta River basin within Ghana: Oti River, White Volta River, and Afram River. We then collected broodstock from the rivers and bred them to obtain fingerlings for a laboratory experiment in aquarium tanks. We assessed the growth performance of fingerlings from the respective *O. niloticus* wild populations using a factorial design with three factors (temperature, dissolved oxygen and salinity) with two levels of each factor (low and high) and three replicates. We also conducted a year-long water quality sampling of the three rivers.

Preliminary results from screening five microsatellite loci showed that all three *O. niloticus* populations studied have high allelic diversity (mean  $\pm$ SD of  $10.1 \pm 5.4$  alleles per locus) and high expected heterozygosity ( $0.75 \pm 0.14$ ). Using the classical  $F_{ST}$  analysis, we found considerable differentiation among the Afram and White Volta ( $F_{ST} = 0.049$ ), Afram and Oti ( $F_{ST} = 0.027$ ) and White Volta and Oti ( $F_{ST} = 0.054$ ). The genetic results suggested that these populations are currently not bottlenecked and are a good resource stock for developing aquaculture strains. Results from the laboratory growth studies showed that overall, the Oti River population tended to grow faster under the “ideal” conditions (low or normal ambient temperature, high dissolved oxygen and low salinity). Additionally, the Oti population appeared to be insensitive to a moderate increase in temperature by showing comparable growth under both the normal and high experimental temperatures. The water quality sampling revealed water temperature variations along the latitudinal gradient in Ghana, with the Oti River exhibiting significantly higher temperatures compared to the other rivers. The results from the growth studies coupled with the water quality sampling suggested that at least the Oti population might already be adapted to high temperature environments, and could be a focal population for developing a regional aquaculture strain with high growth performance under high temperature conditions.

**GENE EXPRESSION ANALYSIS OF ANTIMICROBIAL PEPTIDES DURING LARVAL REARING AND GROW-OUT OF MEAGRE (*Argyrosomus regius*)**

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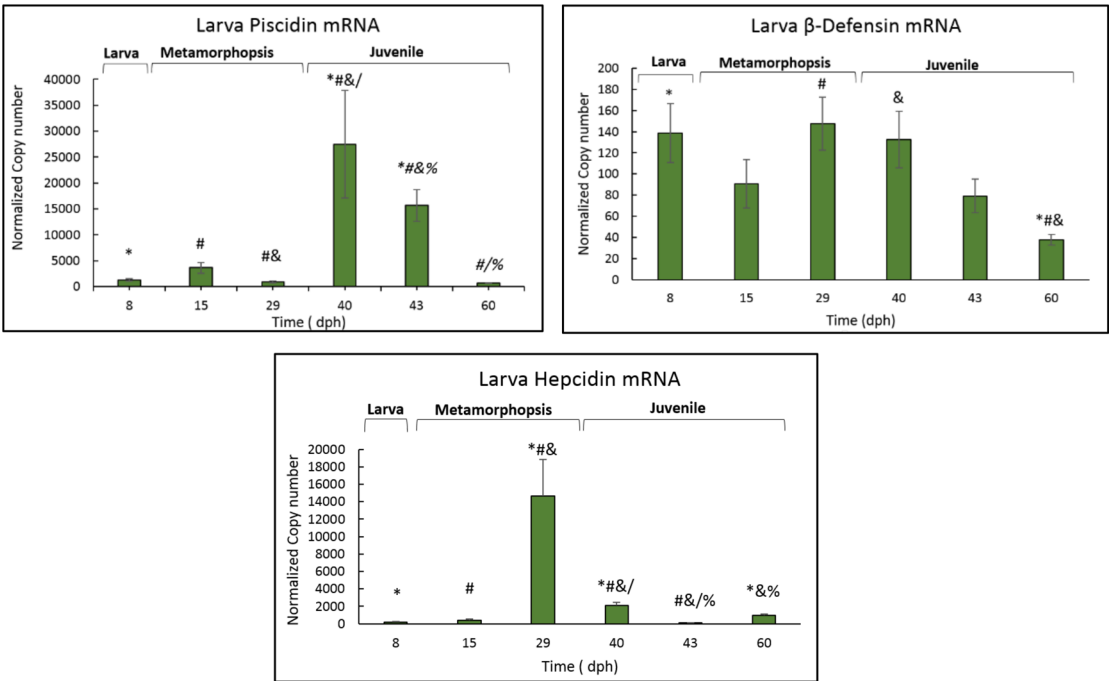
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As part of a larger project funded by the European Union (DIVERSIFY), there are efforts underway to domesticate *Argyrosomus regius* to provide more diversified aquaculture for the European agro-alimentary industrial sector. Among first steps in this ongoing process, are investigations into the functional capacities of the immune system and the chronology of its development during on-growing. This information will aid in understanding the immune functional capacities of fish during grow-out and aid in management of disease outbreaks when they occur.

For this purpose, we have developed a panel of 33 gene expression assays that include 5 endogenous control genes and 28 genes related to various aspects of immune function (innate, humoral, inflammatory response). Herein we present data on the development of the immune capacity supported by antimicrobial peptides (AMPs).

In the case of all three AMPs studied ( $\beta$ -defensin, piscidin, hepcidin), expression was evident as early as 8 dph. A major transition in expression occurs towards the end of metamorphosis that varied in intensity among the three AMPs. In all cases expression diminished after reaching the stage of mature juveniles. Tissue-specific expression (gill, gut, spleen, kidney) was also examined in mature juveniles.

This project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY); IRTA (Research & technology & food agricultura); SENECYT (Secretaria Nacional de Educación Superior, Ciencia, Tecnología e Innovación, Ecuador.)



# INVESTIGATIONS ON QUANTITY AND PROXIMATE QUALITY OF MAGGOTS PRODUCED FROM TWO SUBSTRATES (CATTLE BLOOD AND POULTRY DROPPINGS) CONTAINING VARYING LEVELS OF SAWDUST.

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In an attempt to find alternative protein source for fish feed, this work is one in the series that is aimed at a comparative evaluation of the quantity of maggots grown on different substrates that originate from livestock wastes. An earlier report on this objective was focused on the quantity and quality of maggots produced from poultry droppings, pig dung, cattle dung and cattle blood, without additional fly attractants and without absorbents (Anene *et. al.* 2013). This particular attempt will emphasis on the inclusion of various levels of an absorbent (saw dust) to evaluate the quantity of maggot produced from cattle blood and poultry droppings.

The experiment involved growing maggots on two substrates (cattle blood and poultry droppings) treated with three different levels of sawdust in an open space under a roof at ambient temperature of 30°C. 50kg each of substrates were used per treatment. All data were subjected to analysis of variance using 2 x 3 factorial in completely randomized design.

The wet and dry weight of maggots produced from poultry droppings and cattle blood, at three levels of sawdust inclusion are presented in Table 1.

These results showed that there were significant differences ( $p>0.05$ ) in the weights of maggots (wet and dry) produced from the two substrates. The crude protein level was 43.4% in maggots produced in poultry droppings at 0% level of inclusion of sawdust and 43.2% in maggots produced in poultry dropping at 10% level of inclusion of sawdust. The crude protein content of maggots produced in poultry dropping at 20% level of inclusion of sawdust was 44.1%. Fat content ranges from 8.55 – 8.97% in poultry droppings and 8.65 – 9.17% in cattle blood. Crude fibre content of maggots produced in this study was generally below 2%.

**Table 1:** Wet and dry weights of maggot produced from poultry droppings and cattle blood at varying levels of saw dust inclusion

| Treat<br>ment | Wet<br>weight (g<br>per kg) | Dry<br>weight<br>(g per kg) |
|---------------|-----------------------------|-----------------------------|
| <b>T1</b>     | 147.63 <sup>a</sup> ±39     | 25.32 <sup>b</sup> ±2.74    |
| <b>T2</b>     | 167.91 <sup>a</sup> ±58     | 32.84 <sup>a</sup> ±16.9    |
| <b>T3</b>     | 113.30 <sup>ab</sup> ±3     | 25.09 <sup>b</sup> ±7.49    |
| <b>T4</b>     | 68.67 <sup>b</sup> ±16.     | 14.37 <sup>c</sup> ±4.45    |
| <b>T5</b>     | 152.67 <sup>a</sup> ±24     | 35.47 <sup>ab</sup> ±3.38   |
| <b>T6</b>     | 145.80 <sup>a</sup> ±36     | 29.06 <sup>ab</sup> ±16.5   |
| SEM           | 10.70                       | 2.94                        |

Means in the same column with different superscripts are significant different ( $P<0.05$ ).

**Table 2:** Proximate compositions of maggots produced from cattle blood and poultry dropping at three levels inclusion of sawdust.

|     | Treatments         |                    |                    |                    |                    |                     |      |
|-----|--------------------|--------------------|--------------------|--------------------|--------------------|---------------------|------|
|     | T1                 | T2                 | T3                 | T4                 | T5                 | T6                  | SEM  |
| MC  | 41.2 <sup>a</sup>  | 41.57 <sup>b</sup> | 40.74 <sup>c</sup> | 39.88 <sup>b</sup> | 37.31 <sup>d</sup> | 37.76 <sup>c</sup>  | 0.21 |
|     | ±0.61              | ±0.15              | ±0.14              | ±0.11              | ±0.06              | ±0.21               |      |
| Ash | 4.75 <sup>c</sup>  | 4.93 <sup>b</sup>  | 4.93 <sup>b</sup>  | 4.56 <sup>d</sup>  | 5.63 <sup>a</sup>  | 5.63 <sup>a</sup>   | 0.12 |
|     | ±0.06              | ±0.5               | ±0.1               | ±0.06              | ±0.06              | ±0.06               |      |
| CF  | 1.62 <sup>d</sup>  | 1.63 <sup>d</sup>  | 1.68 <sup>b</sup>  | 1.68 <sup>b</sup>  | 1.66 <sup>b</sup>  | 1.77 <sup>a</sup>   | 0.01 |
|     | ±0.01              | ±0.01              | ±0.01              | ±0.01              | ±0.01              | ±0.01               |      |
| Fat | 8.97 <sup>b</sup>  | 8.67 <sup>c</sup>  | 8.55 <sup>d</sup>  | 8.65 <sup>cd</sup> | 9.17 <sup>a</sup>  | 8.87 <sup>b</sup>   | 0.05 |
|     | ±0.01              | ±0.06              | ±0.02              | ±0.01              | ±0.06              | ±0.06               |      |
| CP  | 43.40 <sup>d</sup> | 43.20 <sup>d</sup> | 44.10 <sup>c</sup> | 45.23 <sup>b</sup> | 46.23 <sup>a</sup> | 45.83 <sup>ab</sup> | 0.28 |
|     | ±0.64              | ±0.06              | ±0.06              | ±0.06              | ±0.06              | ±0.15               |      |

Means in the same column with different superscripts are significant different ( $P<0.05$ ). CF=Crude Fiber, CP=Crude Protein, MC=Moisture Content.



## ASSESSMENT OF THE GROWTH PERFORMANCE AND PROXIMATE QUALITY OF FINGERLING OF *Clarias gariepinus* (BURCHELL, 1822) FED DIETS CONTAINING VARYING LEVELS OF ONION POWDER (*Allium cepa*) AS A PHYTO-ADDITIVE

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Plants could be widely used as feed additives to enhance the efficiency of feed utilization and animal performance (Levic *et al.* 2008). Olfactory feed ingredients enhance growth through the ability to act as feed enhancers (Adams, 2005). With the shift away from synthetic drugs, the use of medicinal herbs as an alternative to antibiotic growth promoters in fish feeding is becoming widely acceptable (Adedeji *et al.*, 2008). This study was carried out to assess the use of onion powder as a phyto-additive in the diet of fingerlings of *C. gariepinus*. The emphasis of this study amongst others was to determine the growth performance and fillet quality of *C. gariepinus* fed on diet containing various levels of onion powder.

A total of hundred (100) fingerlings of *C. gariepinus* were used for the experiment. After acclimatization, the fingerlings randomly sorted, weighed and stocked at the rate of 5 fingerlings per tank and were fed five (5) treatments diets (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> containing 0, 0.5, 1, 2 and 3% levels of onion powder respectively with fifteen replicates each per treatment. The fish were fed to satiation daily at 7:00 hours and 18:00 hours. The experiment was a complete randomized design (CRD). Growth performance of *C. gariepinus* fed experimental diets were evaluated in terms of body weight gain (g), mean daily body weight gain (g) and specific growth rate (Table 1).

After 60 days, weight gain of fish ranged from 90.43±11.23g to 104.57±3.60g and specific growth rate ranged from 0.93±0.04% to 1.02±0.20% per day. Changes in these parameters were not significantly affected by dietary concentrations of onion powder. Crude protein levels in fish fillets fed on diets containing different levels of onion powder is presented in Table 2. Crude protein levels ranged from 22.5±0.06% in fish fed on T<sub>2</sub> to 22.87±0.06% in fish fed on diet T<sub>4</sub>. There were significant differences (P<0.05) in the crude protein contents as 22.87% and 22.80% were recorded for T<sub>4</sub> and T<sub>5</sub> respectively. This may be due to better utilization of free amino acids in diets with higher levels of onion compared with control. Inclusion of onion powder up to 2% resulted in the highest moisture content of 32.4%.

Table 1: Effect of levels of inclusion on growth performance of juvenile *C. gariepinus*.

|                          | Diets            |                  |                  |                  |                  |
|--------------------------|------------------|------------------|------------------|------------------|------------------|
|                          | T <sub>1</sub>   | T <sub>2</sub>   | T <sub>3</sub>   | T <sub>4</sub>   | T <sub>5</sub>   |
| Mean initial body weight | 34.13<br>±1.70   | 35.03<br>±1.70   | 31.93<br>±10.72  | 34.57<br>±0.81   | 33.87<br>±2.15   |
| Mean final body weight   | 134.30<br>±17.35 | 125.47<br>±17.19 | 125.07<br>±17.49 | 133.83<br>±22.47 | 138.53<br>±5.86  |
| Mean body weight gain    | 100.03<br>±16.88 | 90.43<br>±11.23  | 93.13<br>±13.34  | 100.27<br>±23.96 | 100.27<br>±23.96 |
| Mean daily weight gain   | 1.67<br>±0.28    | 1.55<br>±0.22    | 1.55<br>±0.22    | 1.67<br>±0.39    | 1.667<br>±0.39   |
| Specific growth rate (%) | 0.99<br>±0.09    | 1.02<br>±0.20    | 1.02<br>±0.20    | 0.97<br>±0.15    | 0.97<br>±0.15    |

Table 2: Proximate composition of fillets of fish fed to containing various levels of onion powder.

|     | T <sub>1</sub>              | T <sub>2</sub>              | T <sub>3</sub>               | T <sub>4</sub>              | T <sub>5</sub>              |
|-----|-----------------------------|-----------------------------|------------------------------|-----------------------------|-----------------------------|
| Ash | 15.77 <sup>C</sup><br>±0.06 | 16.00 <sup>a</sup><br>±0.00 | 15.33 <sup>d</sup><br>±0.06  | 15.90 <sup>b</sup><br>±0.00 | 16.04 <sup>a</sup><br>±0.01 |
| CF  | 0.47 <sup>C</sup><br>±0.01  | 0.51 <sup>b</sup><br>±0.01  | 0.50 <sup>b</sup><br>±0.01   | 0.53 <sup>a</sup><br>±0.01  | 0.51 <sup>b</sup><br>±0.01  |
| Fat | 0.58 <sup>b</sup><br>±0.01  | 0.56 <sup>C</sup><br>±0.01  | 0.54 <sup>d</sup><br>±0.01   | 0.53 <sup>d</sup><br>±0.01  | 0.62 <sup>a</sup><br>±0.01  |
| CP  | 22.70 <sup>C</sup><br>±0.00 | 22.57 <sup>d</sup><br>±0.06 | 22.80 <sup>bc</sup><br>±0.06 | 22.87 <sup>a</sup><br>±0.06 | 22.80 <sup>a</sup><br>±0.00 |
| MC  | 31.05 <sup>C</sup><br>±0.02 | 31.80 <sup>b</sup><br>±0.00 | 31.70 <sup>b</sup><br>±0.06  | 32.50 <sup>a</sup><br>±0.06 | 31.03 <sup>c</sup><br>±0.06 |
| CHO | 29.46 <sup>a</sup><br>±0.08 | 28.60 <sup>c</sup><br>±0.04 | 29.13 <sup>b</sup><br>±0.06  | 27.70 <sup>d</sup><br>±0.12 | 29.00 <sup>t</sup><br>±0.05 |

\*abcd: Means on the same row with different superscripts are significantly different (P<0.05). CF=Crude Fiber, CP=Crude Protein, MC=Content.

## **HETEROGENEOUS PREFERENCES, INFORMATION, AND KNOWLEDGE FOR ORGANIC FISH DEMAND**

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The past decades have experienced growing demand for ecolabels displaying environmental and sustainability information, with associated price premiums. With growing number of ecolabels in the markets, strategic positioning is required to attract value. Nevertheless, consumer preference for other attributes, for example, local products appears to be overshadowing the value for ecolabels. A suitable communication and education strategy for consumers is warranted to counteract this effect. Using stated choice experiment, we test for the effect of different types of information regarding organic aquaculture production principles on the demand for portion size trout in the German market, while considering other important product attributes. The results indicate that consumers prefer organic produced trout to conventional, and ASC certified trout is seen identical to the conventional product in the status quo market. Influencing the market by providing information for consumers related to feed; stocking density; antibiotics use; and GMO, hormones and synthetic additives while linking to environmental, animal welfare concerns or combination of both reveals that, the preference for environmental is identical to the status quo. Animal health and welfare on the other hand increases the preference level and hence, the perceived value. Combination of environmental and animal welfare information shows a decrease from the animal welfare scenario, an indication that too much information claims on what ecolabel represents does overwhelm consumers. The preference for ecolabel is however, found to be inferior to the country of origin, with the highest value attributed to local production from Germany.

## PRODUCTION OF MICROALGAE FOR SHRIMP LARVICULTURE

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Shrimp culture in Nigeria is not yet well developed despite the abundance of the species in the marine waters. This has been attributed to lack of sustainable production of microalgae both in quality and quantity for shrimp larviculture. A study was conducted at the microalgae laboratory of Nigerian Institute for Oceanography and Marine Research, Lagos, Nigeria to determine the culture possibility of four local microalgae, *Chaetoceros*, *Skeletonema*, *Isochrysis* and *Chlorella* species. *Chaetoceros*, *Skeletonema*, and *Isochrysis* were cultured in modified Guillard F medium while *Chlorella* was cultured in Conwy medium. The growth pattern of the four microalgae showed very short lag phases indicating that they adapted well to the culture conditions. The exponential phases of all the microalgae were similar occurring in the third day of culture. *Chaetoceros*, *Skeletonema*, *Isochrysis*, and *Chlorella* species reached their stationary phases from the sixth to the ninth day with the highest densities of 7,750,000; 1,105,000; 6,091,000 and 6,510,000 cells/ ml respectively. *Chaetoceros* specie had the highest cell density of 7,750,000cells/ ml at the 7<sup>th</sup> day while *Skeletonema* had the lowest density of 101,500 cells/ml at the 13<sup>th</sup> day. The study showed that the microalgae species can grow well and be maintained in the laboratory through continuous sub-culturing. Successful mass cultures of these species indicate that hatchery production of shrimp and prawn larvae is feasible in Nigeria.

# ISOLATION OF NOVEL MUTANT STRAINS OF *Vibrio parahaemolyticus* FROM PACIFIC WHITE SHRIMP *Penaeus vannamei* IN LATIN AMERICA THAT CONTAINS AHPND *PIR A* AND *B* GENES BUT DOES NOT CAUSE ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND)

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The acute hepatopancreatic necrosis disease (AHPND) is an emerging pathogen that affects cultured shrimp *Penaeus vannamei* in several SE Asian and Latin American countries. The causative agents of AHPND have been *Vibrio* sp. including *Vibrio parahaemolyticus*, *V. campbellii* and *V. harveyi*. In this study, we found two isolates (R13 & R14) of *V. parahaemolyticus* that tested positive for AHPND by PCR. Since this finding was the first positive result of AHPND in an AHPND-free area, it was necessary to confirm their pathogenicity through an experimental infection. Juvenile *Penaeus vannamei* were challenged by an immersion method with the two isolates of *V. parahaemolyticus* (R13 and R14), and an AHPND causing *V. parahaemolyticus* strain A3 was used as a positive control. The bacterial doses used for the challenge was  $6.6 \times 10^5$  CFU ml<sup>-1</sup>. Final survival in the treatment tanks (R13 and R14) at termination were 100% in both cases whereas the final survival in the positive control (strain A3) tank was 0% at 24 hr post-infection. AHPND was not detected in the surviving shrimp for the R3 and R4 isolates analyzed by PCR.

To determine the survival of *P. vannamei* shrimp challenged with the R13 & R14 isolates, the *Pir A* and *B* loci in the plasmid DNA of these two isolates were sequenced. Sequence data revealed that the strain R13 is a *Pir A* deletion mutant (*PirA*<sup>-</sup>). For the R14 isolates, the entire *Pir A* and *Pir B* were present, although no mortality was found. A further sequencing of the upstream region of the *Pir A* locus was carried out. An insertion of approximately 4000 bp was found indicating that the isolate R14 is an insertion mutant at a location upstream of *Pir A* and *B* genes (*PirAB*<sup>+</sup>). These findings suggest the need to confirm the presence of bacteria-causing-AHPND in samples that test positive by PCR even samples in which the whole *Pir A* and *B* genes are found. Efforts are underway to sequence the entire genome as well as the plasmid DNA of both R13 and R14 isolates to further elucidate the genetic changes in these isolates compared to the AHPND-causing *V. parahaemolyticus* strain A3.

***Enterocytozoon hepatopenaei* (EHP) IS A RISK FACTOR FOR ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) AND SEPTIC HEPATOPANCREATIC NECROSIS (SHPN) IN THE PACIFIC WHITE SHRIMP *Penaeus vannamei***

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The hepatopancreatic microsporidian *Enterocytozoon hepatopenaei* (EHP) is an emerging pathogen that affects cultured shrimp *Penaeus vannamei* in several SE Asian countries including China, Vietnam, Thailand, Indonesia, India and Malaysia. EHP infections are often accompanied by opportunistic infections of *Vibrio* spp. Laboratory challenges and a case control study were used to determine the effects of EHP infection on two *Vibrio* diseases, acute hepatopancreatic necrosis disease (AHPND) and septic hepatopancreatic necrosis (SHPN). For the effect of EHP on AHPND in 2 laboratory challenge tests in which EHP-infected and healthy shrimp (control group) were challenged with  $2.4 \times 10^5$  CFU/ml of AHPND-causing *Vibrio parahaemolyticus*. The first showed that EHP-infected shrimp exhibited higher mortalities (44 and 60%) than the healthy shrimp (0 and 18%). In the second, their pathological effects were compared during the first 12 hours post infection: 57% of the EHP-infected shrimp displayed severe hepatopancreas necrosis and sloughing, features characteristic of AHPND infection while only 11% of control group showed histopathological lesions. These results indicate that EHP-infected shrimp have a higher susceptibility to AHPND infection. For the effect of EHP on the SHPN, we reviewed the histopathology of samples collected where EHP is endemic and a case control study was carried out to determine the association between SHPN and the EHP. We compared individual shrimp displaying histological signs of SHPN and the shrimp from the same ponds without signs of SHPN. A strong association was found between the presence of SHPN-affected shrimp and the presence of EHP infection, indicating that shrimp with EHP were more likely to be affected by SHPN than the controls. These findings suggested that EHP infection is a risk factor for AHPND and SHPN.

## MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*) OFFSHORE PRODUCTION AT SOUTH PORTUGAL

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Portugal mainland has a coastline of 943 km in continental region. The large exclusive economic zone covers about 1,683,000 km<sup>2</sup>, which includes a potential area for offshore farming mussel of about 2130 km<sup>2</sup>. The southern coast of the Algarve is sheltered from the prevailing conditions from the North Atlantic with significant wave height being generally less than 1 m. Winter storms and SE shore may cause strong sea disturbance, however waves height exceeding 3 m rarely occur.

Mussel cultivation is carried out in series of semi-submerged longlines with an average length of 400 m. Each longline is composed by 250 headlines, with 12 m length and a distance of 1 -1.5m between. The headlines are set about 5 m below the surface. The position and buoyancy is maintained by a system of and anchors (fig.1). Each headline represents an annual average production of 100 kg of mussel.

The mussel produced on Algarve offshore has an average growth rate of 4.36 mm (total shell length) per month (fig.2). The commercial size (50 mm) is reached when mussel has around 1 year old. The condition index and fatty acid profile varies seasonally depending on temperature, food availability and gametogenic cycle. In general there is significant decrease during the winter, recovering throughout the spring. After the spawning period (early spring) the condition index drops back, recovering later during the summer months.

Like the condition index, the total acid value reaches a maximum value of 74.32 mg/g in the early autumn (October) falling to 44.87 in January. The levels of  $\omega$ -3PUFA are relatively high ranging from 21.65 to 32.91 mg/g.

Despite the good growth rates and valuable nutritional quality of the mussel, the Algarve offshore production presents however some limitations such as the high maintenance costs during the winter storms as well as the long periods of capture restrictions due to the high concentration of biotoxins, especially DSP (Diarrhetic Shellfish Poison)

Acknowledgment: This research was funded by DIVERSIAQUA project (MAR2020)

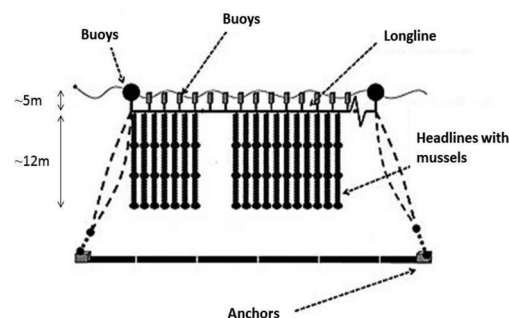


Figure 1- Semi-submerged longline

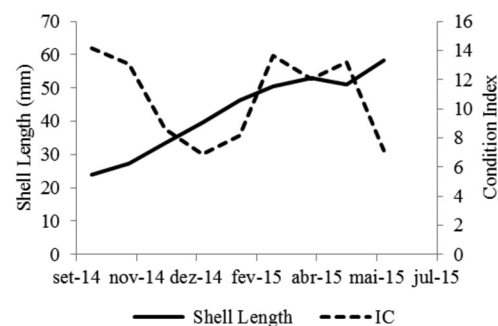


Figure 2- Shell length and IC variation



## THE GROWTH AND SURVIVAL OF CULTURED LARVAE OF *Pecten sulcicostatus*

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The effect of temperature and density on the growth and survival of cultured larvae of *Pecten sulcicostatus*, from D-larvae to pediveliger, was investigated. Rates of growth and survival were determined at temperatures of 12.5, 15.5, 18 and 22 °C. Mean growth rates of larvae increased with temperature ranging from 5.16 to 8.17  $\mu\text{m day}^{-1}$ . These growth rates are similar to those of other commercial scallop species; for example, the reported growth rates of *Nodipecten nodosus*, *Chlamys Hastata* and *Placopecten magellanicus* all fall within the above range. The larvae of *P. sulcicostatus* with the fastest growth rate, i.e. those cultured at 22 °C, typically reached the pediveliger stage 14 days post-fertilization. Larval survival at the experimental temperatures was low with a maximum survival of 12.4% at 22 °C. The mean growth rates of larvae cultured at 22 °C at densities of  $4 \times 10^3$ ,  $10 \times 10^3$  and  $15 \times 10^3$  larvae  $\text{L}^{-1}$  ranged from 6.11 to 6.54  $\mu\text{m day}^{-1}$  with higher growth at the lowest density. Maximum larval survival at these experimental densities was 2.9%. The low survival in all experiments was neither correlated to temperature nor stocking density. Other factors such as sub-optimal food concentrations, system design and water changing techniques need to be investigated to ascertain the causes of low survival.

## A PRELIMINARY STUDY ON OPTIMIZATION OF DIETARY DUCK WEED MEAL IN RAINBOW TROUT JUVENILES

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Aquaculture is the fastest growing food production sector in the world and approximately half of all fish consumed by humans comes from aquaculture. The cost of feed is the major expenditure item for aquaculture producers. The continuous global growth and intensification of aquaculture demand high amounts of fishmeal, the main ingredient that has been used conventionally as the major source of dietary protein due to its well-balanced amino acid profile and palatability. Nevertheless, high amounts of fishmeal from marine sources have become a concern both environmentally and economically. Thus, the aquaculture industry needs to find new locally-produced, low-cost, profitable and environmentally-friendly raw materials for innovative practical aquafeed production.

In the present study, we tested duckweed meal (DM) as an alternative raw material for rainbow trout feed. Fish were fed diets with different level of DM for 8 weeks: 0% (DM0), 8% (DM1), 16% (DM2) and 32% (DM3). At the end of the feeding trial, fish fed DM2 and DM3 diets had significantly lower growth than those fed control diet (DM0) (Fig. 1). Antioxidant defense system and lipid peroxidation was significantly influenced by the dietary treatments ( $P<0.05$ ). The activity of superoxide dismutase and the amount of glutathione decreased with the increasing dietary DM while lipid peroxidation level increased at the same circumstance. Fatty acid profile was significantly affected by the experimental diets ( $P<0.05$ ). Fatty acids such as 16:0 and 18:1n-9 decreased with the increasing dietary DM while 22:6n-3 level increased at the same circumstance. Our study suggested that DM can be used in the diet for rainbow trout juveniles up to 8% without any negative effects. Further studies should focus on possible anti-nutritional factors existing in DM in order to better optimize the dietary level of this raw material.

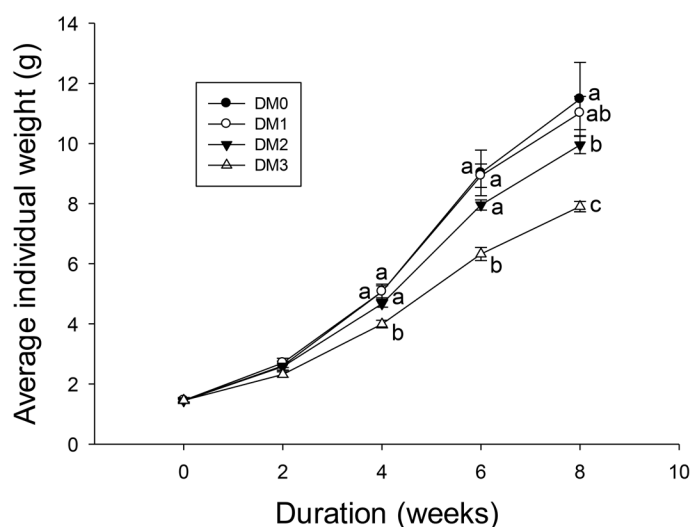


Fig. 1. Growth performance of rainbow trout juveniles fed experimental diets.

## **USE OF PITUITARY EXTRACTS, GNRHA + METACHRORIDE (DAGIN) AND WATER FOR OPTIMISING EGG PRODUCTION, FERTILISATION AND HATCHABILITY OF LARVAE IN INDUCED SPAWNING OF FIRST GENERATION OF *Barbus altianalis* RAISED IN CAPTIVITY**

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In a study to optimize production of viable eggs for improved hatchability during artificial spawning in *Barbus altianalis*, the effect of catfish pituitary extract on induced spawning of both second generation males and females was compared to that of Dagin (GnRHa + metachroride) and water alone in two experiments. In the experiment I the effectiveness of using catfish pituitary extracts was compared with the Dagin and in the experiment II only the females with some running eggs were facilitated to spawn by water running through the tanks. Significant variations in fertilization, hatchability and working fecundity were observed ( $p < 0.05$ ). Fish treated with catfish pituitary extracts performed much better than those treated on Dagin with respect to fertilization rates at 80% ( $U=66.5$ ,  $p < 0.001$ ) and working fecundity at  $2314.40 \pm 882.04$  ( $U=59.5$ ,  $p < 0.05$ ). However, the difference with hatchability was not significant ( $p > 0.05$ ). In experiment II fertilization rates, hatchability and working fecundity were significantly better when fish was striped after 4 hours (100 degree hrs) of running water only than those striped after 10hrs (250 degree hrs). The findings confirmed that catfish pituitary extracts are effective in inducing the *B. altianalis* to spawn. Also the ripe females with some running eggs need not to be induced with any hormone. Hence the cost of spawning the fish could further be reduced only using running water especially in wet seasons when the majority of the fish were found to have ripe running eggs.

## **ANALYSIS OF FISH TRADE IN THE EASTERN CORRIDOR : THE CASE OF CENTRAL UGANDA**

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Fishing in Uganda is one of the few livelihood activities that hold great potential for income generation and poverty reduction especially among the individuals or households living in both near and far away from water resources in Uganda. However, the fisheries subsector has of recent suffered a setback due to dwindling fish supplies from the lakes and rivers and an infant subsector. Compared to other branches of agriculture, fisheries industry has received less research, especially in the area of socioeconomics, particularly marketing. This study set out to contribute to this knowledge gap. The main objective of the study was to examine the market structure, conduct and performance of aquaculture subsector.

The study was conducted in the districts of Mpigi, Mukono, Wakiso and Kampala -the major fish producing and consuming markets in Uganda. A pretested structured questionnaire was used to collect data from a randomly selected sample of 232 of fish traders. The collected data were then coded and analyzed by employing SPSS and STATA computer programs. Both descriptive and econometric methods were used to analyze the data. A Structure conduct and Performance (SCP) framework was adopted; a concentration ratio and Hirschman Index (HHI) were computed.

The results show that fish trade is dominated by males at wholesale (26.7%) level while female dominate (38.4%) at retail level. The results also show that there are significant differences between the two gender categories. Location had significant influence on fish trade and urban area was more favored. The computed Concentration ratio of 0.799 implied that a few traders dominate the fish market. The computed HHI index was 0.5 reinforcing the preceding findings. Thus, there seems to be limited competition among fish traders leading to inefficiencies. Econometric results revealed that the significant factors affecting marketing efficiency include: gender, transport costs, selling price and the district of origin for fish.

There is a need for policies geared towards improving gender relation, infrastructure in terms of roads to reduce transport costs and improving market information relayed to the farmer. To improve market structure, efforts to reduce barriers such as high taxes to market entry should be made.

TRICHODINIDS OF NILE TILAPIA *Oreochromis niloticus* IN BRAZIL: FIRST RECORD OF *Trichodinella* sp.

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Massive occurrence of trichodinids is frequently accompanied by a serious disease in fish farm. This study characterized morphologically species of trichodinids from gills and body of Nile tilapia cultivated in Brazil. Dried slides with parasites were impregnated with silver nitrate (2%). Morphometric characters (Table 1) and schematic drawing of the denticles were taken from photomicrographs (Figure 1) made from the slides. The measurements were arranged and presented in micrometers (µm): mean ± standard deviation.

In the present study, we found 7 species of trichodinids parasitizing the gills, being four of the genera *Trichodina* Ehrenberg, 1838; one *Tripartiella* Lom, 1959; one *Paratrachodina* Lom, 1963 and one *Trichodinella* Srámek-Husek, 1953 (Table 1). On the body surface, three specimens from genera *Trichodina* were identified (Table 1).

All parasites were recently identified parasitizing fish in Brazil, except for *Trichodinella* sp.. This is the first record of parasites of this genus in this region.

Table 1. Morphometric characters of trichodinids (measurements in micrometers).

| Trichodinids species             | Site  | Body diameter | Border membrane | Adhesive disc | Denticulate ring | Central circle | N of central ridges | Span         | Ray         | Central part | Blade       | Denticle length | N of denticles | Pins per denticle |
|----------------------------------|-------|---------------|-----------------|---------------|------------------|----------------|---------------------|--------------|-------------|--------------|-------------|-----------------|----------------|-------------------|
| <i>Trichodina centrostrigata</i> | Gills | 55.73 ± 5.02  | 3.71 ± 0.84     | 49.03 ± 5.31  | 26.04 ± 2.89     | -              | 13.22 ± 1.93        | 12.82 ± 1.94 | 4.57 ± 0.98 | 2.42 ± 0.54  | 5.73 ± 0.94 | 4.51 ± 0.37     | 27.71 ± 1.00   | 9.33 ± 1.15       |
| <i>Trichodina migala</i>         | Gills | 76.01 ± 5.66  | 5.67 ± 0.50     | 64.71 ± 6.83  | 40.79 ± 5.50     | -              | -                   | 18.48 ± 2.02 | 8.30 ± 1.59 | 3.43 ± 0.81  | 6.56 ± 0.87 | 8.53 ± 0.48     | 27.00 ± 1.41   | 10.00 ± 0         |
| <i>Trichodina heterodentata</i>  | Gills | 70.45 ± 6.06  | 5.43 ± 0.53     | 59.61 ± 5.58  | 39.09 ± 6.99     | -              | -                   | 17.64 ± 1.64 | 9.61 ± 1.33 | 2.46 ± 0.27  | 5.51 ± 0.52 | 9.00 ± 1.03     | 23.90 ± 2.08   | 10.40 ± 1.14      |
| <i>Trichodina compacta</i>       | Gills | 53.09 ± 2.20  | 4.61 ± 0.53     | 44.35 ± 2.09  | 27.13 ± 2.13     | 13.11 ± 1.66   | -                   | 11.80 ± 0.87 | 5.38 ± 0.50 | 2.30 ± 0.33  | 4.12 ± 0.51 | 8.16 ± 0.51     | 19.00 ± 0.76   | 8.89 ± 1.05       |
| <i>Paratrachodina africana</i>   | Gills | 25.04 ± 2.50  | 1.80 ± 0.19     | 21.24 ± 2.42  | 12.42 ± 1.88     | -              | -                   | 5.82 ± 0.48  | 1.88 ± 0.33 | 0.54 ± 0.10  | 3.33 ± 0.27 | 2.42 ± 0.39     | 22.17 ± 1.10   | -                 |
| <i>Tripartiella orthodens</i>    | Gills | 27.36 ± 1.35  | 2.50 ± 0.31     | 22.36 ± 1.34  | 10.88 ± 0.88     | -              | -                   | 7.42 ± 0.73  | 1.91 ± 0.23 | 0.54 ± 0.06  | 4.87 ± 0.60 | 2.57 ± 0.33     | 21.44 ± 1.01   | 5 ± 0             |
| <i>Trichodinella</i> sp.         | Gills | 22.94 ± 2.38  | 2.04 ± 0.38     | 21.65 ± 2.84  | 12.01 ± 1.72     | -              | -                   | 4.37 ± 0.35  | 0.56 ± 0.08 | 1.3 ± 0.15   | 2.34 ± 0.47 | 2.21 ± 0.28     | 21 ± 1.41      | -                 |
| <i>Trichodina compacta</i>       | Body  | 54.61 ± 3.39  | 4.57 ± 0.47     | 45.41 ± 3.30  | 28.19 ± 2.71     | 13.75 ± 1.73   | -                   | 11.86 ± 0.73 | 4.91 ± 0.55 | 2.51 ± 0.32  | 4.43 ± 0.35 | 8.23 ± 0.56     | 19.29 ± 0.96   | 8.32 ± 0.48       |
| <i>Trichodina heterodentata</i>  | Body  | 70.10 ± 6.15  | 5.37 ± 0.70     | 59.41 ± 6.14  | 37.61 ± 3.92     | -              | -                   | 17.90 ± 1.41 | 9.60 ± 1.05 | 2.66 ± 0.32  | 5.55 ± 0.62 | 9.44 ± 1.06     | 23.67 ± 1.03   | 10.76 ± 0.90      |
| <i>Trichodina magna</i> **       | Body  | 91.86         | 7.86            | 75.79         | 49.56            | -              | -                   | 24.41        | 13.10       | 3.63         | 7.44        | 10.09           | 29.00          | 9.00              |

\*\* Only one parasite of this specie were found in the present study

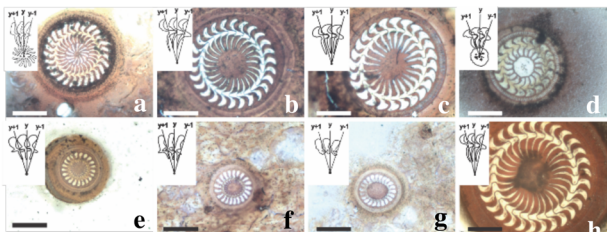


Figure 1. Silver impregnated adhesive discs and schematic drawing of *Trichodina centrostrigata* (a); *T. migala* (b); *T. heterodentata* (c); *T. compacta* (d); *Paratrachodina africana* (e); *Tripartiella orthodens* (f); *Trichodinella* sp. (g) and *Trichodina magna* (h) obtained in the present study. Scale bars: 20 µm.

## THIAMPHENICOL: *IN VITRO* ACTIVITY AGAINST BACTERIA PATHOGENIC TO FISH

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Thiamphenicol (TAP) is a structural analogue of chloramphenicol and have similar antibacterial spectrum. TAP is widely used as an antibacterial both in human and veterinary medicine and it has been proposed for the treatment of vibriosis and pasteurellosis in fish farm. In the present study we investigated the TAP *in vitro* activity against 30 strains of bacteria isolated from different freshwater fishes during disease outbreaks in Brazil. The bacteria identities were confirmed by 16S rRNA gene sequencing, being 1 *Aeromonas caviae*, 8 *Aeromonas hydrophila*, 2 *Aeromonas jandaei*, 1 *Aeromonas veronii*, 2 *Citrobacter freundii*, 2 *Enterococcus casseliflavus*, 3 *Lactococcus garviae*, 2 *Pseudomonas fulva*, 6 *Streptococcus agalactiae* and 3 *Vibrio* spp.. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth microdilution protocol recommended by the Clinical and Laboratory Standards Institute (CLSI/NCCLS). There are no published quality control (QC) ranges available for testing of TAP at 28°C. Thus, *Escherichia coli* ATCC 25922 QC ranges have been established at 28°C using the CLSI recommended procedures. Reference strain *Aeromonas hydrophila* ATCC 7966 was included in the analyses for comparative purposes. All isolates were recovered and cultured prior to being analyzed using specific medium to each strain. TAP was commercially obtained from Sigma-Aldrich and 95% ethanol was used as a solvent to prepare 20480 µg TAP mL<sup>-1</sup> antimicrobial stock solution. Twenty microliters of the antimicrobial stock solution was transferred to the 1<sup>st</sup> well of a standard 96 well format plates containing 180 µL medium and then serial 2-fold dilution was made (range of concentrations: 1024 – 0.0078 µg TAP mL<sup>-1</sup>). MICs were done in triplicate and the endpoints were determined by using the standard MIC method and tetrazolium method. Three wells in each MIC test plate were used as a control. Cation-adjusted Mueller-Hinton broth (CAMHB, Difco) was used for susceptibility testing of *A. caviae*, *A. hydrophila*, *A. jandaei*, *A. veronii*, *C. freundii*, *E. casseliflavus*, *L. garviae*, *P. fulva* and *Vibrio* spp.. CAMHB supplemented with 2.5 lysed blood and with 1.5% NaCl was used for susceptibility testing of *S. agalactiae* and *Vibrio* spp., respectively. MIC test plates were incubated at 28°C for 24h.

The MICs and MBCs of TAP for 30 freshwater fish bacterial isolates are summarized in Table 1. TAP demonstrated highly potent antibacterial activity against *Aeromonas* spp., *Lactococcus* spp. and *Vibrio* spp., although in some strains of *Aeromonas* spp. and *Vibrio* spp. it was somewhat less sensitive (>8 µg mL<sup>-1</sup>). Aeromoniosis, lactococcosis and vibriosis are one of the most important diseases in aquaculture worldwide and these findings suggest the potential of TAP as a treatment option for these diseases.

| Strains used (n)                      | Fish specie* | Period of isolation | TAP antibacterial activity (µg mL <sup>-1</sup> ) |             |
|---------------------------------------|--------------|---------------------|---|-------------|
|                                       |              |                     | MIC   | MBC         |
| <i>Aeromonas caviae</i> (1)           | 4            | 2014                | 8   | 8           |
| <i>Aeromonas hydrophila</i> (8)       | 2 and 4      | 2011-2015           | 1 - 32  | 2 - 32      |
| <i>Aeromonas jandaei</i> (2)          | 4            | 2011-2013           | 1 - 2   | 2           |
| <i>Aeromonas veronii</i> (1)          | 2            | 2014                | 1   | 2           |
| <i>Citrobacter freundii</i> (2)       | 1 and 3      | 2012-2015           | 64  | 64 - 256    |
| <i>Enterococcus casseliflavus</i> (2) | 4            | 2010-2011           | 16 - 64   | >128 - >256 |
| <i>Lactococcus garviae</i> (3)        | 3 and 4      | 2011-2012           | 2 - 4   | >8 - >16    |
| <i>Pseudomonas fulva</i> (2)          | 4            | 2011-2012           | 128 - >1024                                       | >512        |
| <i>Streptococcus agalactiae</i> (6)   | 4            | 2014-2016           | 128   | >512        |
| <i>Vibrio</i> spp. (3)                | 2            | 2016                | 2 - 128   | 4 - <512    |

\*Fish species: 1- freshwater angelfish, *Pterophyllum scalare*; 2-pacu, *Piaractus mesopotamicus*; 3- cachara, *Pseudoplatystoma reticulatum* and 4- Nile tilapia, *Oreochromis niloticus*.



QUANTITATIVE SKIN COLOUR AND HAEMATOLOGICAL CHANGES IN TILAPIA *Oreochromis niloticus* TREATED WITH HERBAL EXTRACTS AGAINST *Streptococcus agalactiae* INFECTION

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Infection by *Streptococcus* in tilapia leads to unsightly and unmarketable fish due to spine displacement, abdominal distension, eye/skin haemorrhage as well as ‘pop-eye’. Treatment with herbs is based on immune-modulation via haematological factors that are responsible for fighting infection. The present study sought to understand the multivariate relationship underpinning immunomodulation in tilapia using two herbal extracts (ethanol extracts of garlic (G) and betel leaf (P)) administered in feed at two levels (low(L):GL,PL and high(H):GH,PH) each, as well as controls to elucidate the effects of infection and herbal treatment on skin colour.

Initial feeding of tilapia juveniles lasted 7 days followed by challenge using *S. agalactiae* at concentration of 1 x 10<sup>8</sup> CFU ml<sup>-1</sup>. After 14 days of challenge, blood samples were collected and parameters determined using blood cell automatic cell counter. Skin pigmentation was quantified from digital images passed to Matlab’s image processing feature to obtain CIELab values and subsequently CIEΔE 2000 colour differences.

Herbal treatment significantly accounted for the variability in neutrophils by 54% (Table 1) with significant difference in the values for all treatments. Challenge on the other hand significantly accounted for variation in the neutrophils, lymphocytes and basophils at 51%, 68% and 40% effect sizes respectively. Fish exhibited skin colour differences (Table 2) based on the challenge as well as treatment received. Clustering of treated fish produced 4 groups via k-means algorithm (Figure 1). Fish that were not subjected to challenge clustered separately from all the other fish. Results of the present study indicate a strong effect of herbal treatment on neutrophils while challenge affected two other WBC components in addition. Herbal treatment and challenge also affected skin colour relative to unchallenged fish with clusters based on treatment.

| WBC Differentials (%) | Herbal Treatment  |       |      |
|-----------------------|-------------------|-------|------|
|                       | $\chi^2$          | p     | r    |
| Neutrophils           | 14.73             | 0.012 | 0.54 |
| Monocytes             | 0.85              | 0.974 | 0.03 |
| Lymphocytes           | 4.45              | 0.487 | 0.16 |
| Basophils             | 8.67              | 0.123 | 0.32 |
|                       | Challenge Feeding |       |      |
|                       | W                 | p     | r    |
| Neutrophils           | 155.5             | 0.008 | 0.51 |
| Monocytes             | 117.0             | 0.363 | 0.25 |
| Lymphocytes           | 21                | 0.000 | 0.68 |
| Basophils             | 128.5             | 0.048 | 0.40 |

Table 2 CIEΔE Colour Difference Matrix of Tilapia skin

|    | Reference |        |        |       |       |        |
|----|-----------|--------|--------|-------|-------|--------|
|    | GH        | GL     | PH     | PL    | C1    | C2     |
| GH |           | 11.996 | 12.867 | 4.742 | 9.439 | 17.196 |
| GL |           |        | 1.925  | 7.687 | 2.703 | 8.516  |
| PH |           |        |        | 8.293 | 4.170 | 6.611  |
| PL |           |        |        |       | 5.430 | 12.583 |
| C1 |           |        |        |       |       | 10.345 |
| C2 |           |        |        |       |       |        |

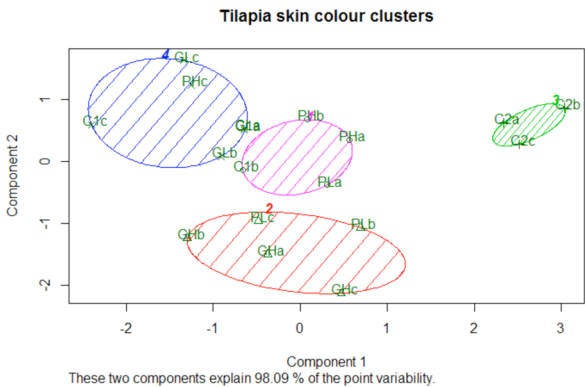


Figure 1 Cluster plot of CIELab skin colour values of tilapia challenged with *S. agalactiae*

## INVESTIGATIONS ON PURIFICATION AND CHARACTERIZATION OF 6-PHOSPHOGLUCONATE DEHYDROGENASE FROM RAINBOW TROUT GILLS AND INHIBITION EFFECTS OF SOME METAL IONS ON THE ENZYME ACTIVITY

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The enzyme 6-phosphogluconate dehydrogenase (6-PGD) (6-PGD; E.C. 1.1.1.44) was purified from rainbow trout gills. Purification was performed in 2 steps: homogenate preparation and 2',5' ADP Sepharose 4B affinity chromatography. The enzyme 6-PGD with 10.268 EU/mg specific activity was purified at 401-fold with a yield of 40%. Enzyme activity was measured spectrophotometrically at 340 nm. The optimum pH, optimum ionic intensity, optimum temperature and stable pH for the enzyme were 8.0, 0.4 M, 50°C and 8.0, respectively.  $K_M$  and  $V_{max}$  values were determined as 0,89  $\mu$ M and  $V_{max}$  0,331 EU/mL for NADP<sup>+</sup> and as 0.16 mM  $V_{max}$  and 1.52 EU / mL for 6-PGA, respectively. Also, inhibition effects of some metals on 6-PGA and  $K_i$  values were determined.  $K_i$  values for the metals were determined as to be: Ag<sup>+</sup> 0.082 $\pm$ 0.017 Hg<sup>2+</sup> 0.071 $\pm$ 0.012 Fe<sup>3+</sup> 0.0095 $\pm$ 0.0007 Mn<sup>2+</sup> 0.028 $\pm$ 0.004, Cu<sup>2+</sup> 0.0037 $\pm$ 0.00089 Mg<sup>2+</sup> 0.25 $\pm$ 0.06 Zn<sup>2+</sup> 0,023 $\pm$ 0,005 Ta<sup>+</sup> 0,0031 $\pm$ 0,0013 Ni<sup>2+</sup> 0.015 $\pm$ 0.019 Co<sup>2+</sup> 0.003 $\pm$ 0.0019, respectively.

## CURRENT SITUATION OF AQUACULTURE PRODUCTION IN MUĞLA PROVINCE, TURKEY

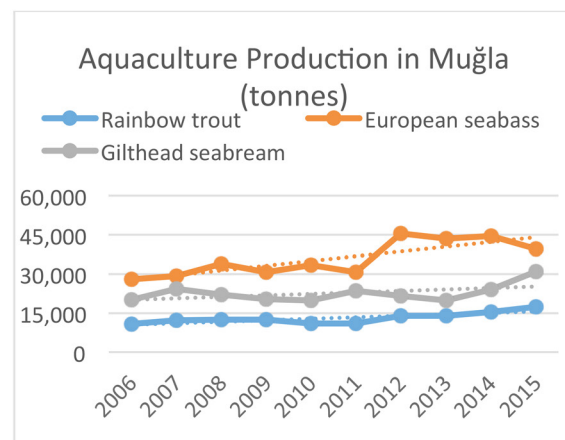
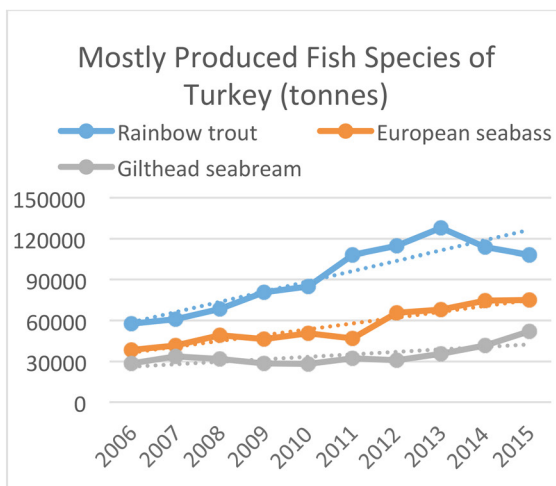
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Increasing of interest in the aquaculture sector in Turkey has been rapidly caused physical growth in a short time. In Turkey, the most produced species is Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) with 108.038 tones in the total production (240.334 tones) in 2015. The second most produced species is European seabass (*Dicentrarchus labrax* Linnaeus, 17858) with 75.164 tones and the third most produced species is Gilt-head sea bream (*Sparus aurata* Linnaeus, 1758) with 51.844 tones. 37,4% of the total aquaculture production and, 55.5% of production of Gilt-head sea bream and European seabass have been produced in Muğla province in 2015.

In Muğla province, there have been 102 cage net facilities, 166 earthen pond facilities, 84 Rainbow trout facilities and the total production of these facilities were respectively 89.160; 9.256,5; 14.581,5 tones. Additionally, there have been 7 marine fish production hatcheries which have 305.500.000 number/year production capacity and 10 aquaculture processing plants which have 109.000 tones. In Muğla province where aquaculture production is highly concentrated, 40.751 tones of aquaculture products have been exported and 261.828.733 \$ income has been obtained.

Small-scale aquaculture facilities in rural areas provide employment opportunities and sustainability of the population density in developing countries. Investments in the aquaculture sector are increasing each passing day that are expected to have an important role in meeting the growing population's nutritional needs in the future. Making investments with good planning without neglecting of social, economic and ecological interactions is crucial for the sustainable management of aquaculture sector. As a consequence, countries which have aquaculture potential, need to take into account the sustainable management of the current production.



## PROXIMATE COMPOSITION OF *Litopenaeus vannamei* JUVENILES GROWN IN A BFT SYSTEM WITH DIFFERENT DIETS AND C:N RATIOS

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BFT technology increases the efficiency of nutrient recycling, favoring single-cell protein synthesis in intensive closed culture systems. In this work we aimed to evaluate the effect on the proximate composition of *Litopenaeus vannamei* juveniles fed 35 and 40% protein diets, supplied with different amounts of molasses (50 y 65%) to maintain the same environmental C:N ratio (diets BFT<sub>35-50</sub> and BFT<sub>40-65</sub>, C:N ratio 19:1). The 35% diet was supplied also with 65% molasses, to achieve a 21:1 C:N ratio (diet BFT<sub>35-65</sub>).

Shrimps fed the 35% protein diet without molasses served as controls. The feeding experiment lasted 42 days and each treatment was run in triplicate 1 m<sup>3</sup> fiberglass tanks stocked with an initial biomass of 668±2.7 g m<sup>-3</sup> (mean average wet weight 4.4±0.2 g, equivalent to an approximate initial density of 151 shrimps m<sup>-3</sup>). Final samples, analyzed with standard techniques and compared with one-way ANOVA tests, showed in all cases significantly ( $P<0.05$ ) lower values in control shrimps. The highest protein content was achieved with the BFT<sub>40-65</sub> diet, which gave a significantly better value than BFT<sub>35-50</sub>. Diet BFT<sub>35-65</sub> gave intermediate values. There were no differences in the lipid contents of shrimps of the BFT treatments, and all were consistently higher ( $P<0.05$ ) than control shrimps. Results show that BFT achieves effective nitrogen incorporation into shrimp biomass and that a slight increase of the C:N ratio, such as that of diet BFT<sub>35-65</sub>, may result in a protein content as high as that obtainable with a higher protein diet.

Table 1. Mean (± standard deviation) proximate composition of the head-on *Litopenaeus vannamei* (wet weight) juveniles fed the experimental diets.

| Diets and C:N ratio | Control<br>(7.1:1)       | BFT <sub>35-50</sub><br>(19:1) | BFT <sub>35-65</sub><br>(21:1) | BFT <sub>40-65</sub><br>(19:1) |
|---------------------|--------------------------|--------------------------------|--------------------------------|--------------------------------|
| Proteins (%)        | 4.47 ± 0.65 <sup>a</sup> | 6.09 ± 0.68 <sup>b</sup>       | 9.13 ± 2.78 <sup>bc</sup>      | 8.03 ± 0.75 <sup>c</sup>       |
| Lipids (%)          | 0.81 ± 0.12 <sup>a</sup> | 1.29 ± 0.09 <sup>b</sup>       | 1.73 ± 0.46 <sup>b</sup>       | 1.42 ± 0.12 <sup>b</sup>       |
| Ashes (%)           | 0.88 ± 0.02 <sup>a</sup> | 1.39 ± 0.10 <sup>b</sup>       | 2.17 ± 0.57 <sup>c</sup>       | 1.83 ± 0.26 <sup>c</sup>       |

Different letters indicate significant differences ( $\alpha=0.05$ , a<b<c).

Supported by projects PROFAPI2014/022, CIBNOR AC0.38, PFCE 2016

## FARMING *Saccharina angustissima*, A UNIQUE KELP, IN THE GULF OF MAINE, USA WITH A FOCUS ON PRODUCTION AND ECOSYSTEM SERVICES

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Locally sourced, high quality sea vegetables, in particular kelp, are sparking consumer interest and demand in the USA. Despite existing challenges such as development of aquaculture systems and infrastructure, many opportunities are becoming available for sea farmers. *Saccharina angustissima*, a unique low intertidal strap-like kelp from Casco Bay, Maine, has desirable culinary traits enabling it to be a novel cultivated crop. This kelp is adapted to withstand extreme hydrodynamic forces, an order of magnitude higher than the subtidal populations of sugar kelp that are usually found in more sheltered locations. In the wild, the kelp blades are up to 4.5 m long and strictly narrow at 1-5 cm wide. Thalli are annuals with peak sorus production in October thru late November. We will present two years of data from the two geographically distinct open-water farms where we began the domestication process. Based on harvest results from two growing seasons, kelp biomass yields were higher than the common sugar kelp, *S. latissima*. Highest yields were achieved with averages of up to  $24.1 (\pm 6.3)$  kg m<sup>-1</sup> of line with a plant density of 400 plants m<sup>-1</sup> of line (Fig.1). The morphological characteristics that make this kelp exceptionally adapted to extreme sea conditions were preserved at the open-water farm sites. Growing it offers a suite of ecosystem services including nutrient bioextraction. Calculations estimating the nutrient bioextraction capability of the domesticated *S. angustissima* kelp biomass harvested in June reveal N removal of 88.7 kg ha<sup>-1</sup> and C removal of 1666.7 kg ha<sup>-1</sup>. These are combined farm site averages based on a hypothetical farm of forty 100 m longlines spaced 2.5 m apart per hectare. This kelp has great potential as an economically valuable sea vegetable in the Gulf of Maine but care must be taken to protect the donor population (Fig.2). Additional work needs to be done to bring this kelp to market.



Figure 1. Strap-like kelp, *Saccharina angustissima* growing in the low intertidal, Maine, USA.

Figure 2. Sea-farmer observing *Saccharina angustissima* harvest.

## TOWARDS BALANCING LAKE-USE AND CULTURE FISHERIES OFF LAKE VICTORIA BEACHES, KENYA: INTEGRATING MAPPING AND SOCIO-ECONOMIC ASSESSMENT OF CAGES

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Mapping of lacustrine aquaculture enterprises and socio-economic assessment of cage farmers operations can be employed as decision support tool in an integrated fashion for fisheries management. We simultaneously mapped and reported the location of cages and characterized socio-economic status of cage farming in Lake Victoria, Kenya. Structured questionnaires and interviews from cage culture farmers generated socio-economic data and management information. Cage culture was found to be a male-dominated activity with the majority of owners aged < 45 years (n = 23; 59%). Siaya County had the highest cage establishments (n = 20) and number (n = 1343). Proximity to Dominion Farm, pioneers of cage culture, as well decreased presence of water hyacinth (*Eichhornia crassipes*) coverage may have contributed to high cage numbers in Siaya County. The only species cultured was *O. niloticus*. Most cage establishments (n = 30; 76%) were located within 200 m from the shoreline, despite demarcation as breeding zones. Total operational costs for a cycle (8 months) amounted to USD 465,250 worth a total production value of USD 8,827,000, indicative of its robust viability within “The Blue Economy” concept. With the increasing number of cages in the lake, there is need for policy and regulations to guide its investment, both to protect local economies through improved business practices and to ensure sustainability for the lake ecosystem.



## **VERTICAL SUBSTRATES IN BIOFLOC SYSTEMS AN INTERMEDIATE INTENSIVE AQUACULTURE SYSTEM**

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The suspension of bioflocs in the water is essential in conventional biofloc systems. This necessitates the use of high energy water mixing, a condition that is often beyond the possibilities of small farmers. An alternative to the use of suspended biofloc systems was developed in the Hitide Seafarms, Tamil Nadu, India, by its owner Boriah Suryakumar.

The methodology is based on hanging plastic shade nets in the water, held by series of floats and anchors, preferably placed in front of a paddle wheel aerator. The vertical substrates adsorb organic detritus, serving as a base for the development of an aquatic biota, similar to that of bioflocs. The presence of the vertical substrates reduce the extent of organic sedimentation onto the bottom of the pond. The sedimentation is replaced by the accumulation of the organic residues within the aerated water layer, this enabling an effective metabolism and recycling of feed waste in the pond. The biological processes in the pond is based upon the addition of molasses so as to maintain a C/N ratio of 10 in order to prevent excessive TAN concentration in the water.

Accumulated experience demonstrates that the placement of the vertical substrates helps in increasing shrimp growth rates, lower sludge accumulation, reduces FCR and reduces diseases occurrence. It seems that the methodology demonstrated in Hitide Seafarms can be used by farmers, as such or as an intermediate system toward higher intensification, Further research and development in the farm is underway.

## **BIOFLOC TECHNOLOGY: PROVEN AND POTENTIAL ADVANTAGES TOWARD FUTURE INTEGRATED AQUACULTURE**

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The aquaculture industry struggles to exist in light of low product prices, increasing costs of inputs and constraints due to environmental, water and land limitations.

Intensive aquaculture systems are relevant to efficiently produce fish and shrimp. However, an intrinsic problem of these systems is the rapid accumulation of feed residues, organic matter and toxic inorganic nitrogen species. Biofloc technology (BFT) systems is a practical solution. Water treatment is based upon developing and controlling heterotrophic bacteria within the culture component, in tandem with nitrifying bacteria and algae. Feed nutrients are recycled, doubling the utilization of protein and raising feed utilization.

The basic concept of BFT is controlling the pond as an integrated system, not separately treating water quality, fish production, zooplankton, disease etc. The bioflocs are an ecological niche containing bacteria, algae, protozoa and zoo-plankton, fed with feed residues and recycling the feed within this niche. Fish feed the bioflocs and harvest them. Bioflocs were shown to improve the immunity of fish and shrimp toward diseases.

Feeding on bioflocs implies economical, natural feed additives and fish health considerations. Presently, research is directed to select biofloc features that provide better health immunity and nutritional values. An intensive direction of research is directed on the search for efficient microbial communities, toward such communities that will optimize cultured animals growth, increase immunity against pathogens and improve nutritive values of the harvested fish.

Another development, to be discussed in a separate presentation, is to develop methodology easing the adsorption of biofloc technology for family farms. We hope this direction will ease intensive aquaculture assimilation in Africa.

## EVALUATION OF GUT EVACUATION RATE IN AFRICAN CATFISH *Clarias gariepinus* FED BENTONITE CLAY AS FEED ADDITIVE

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The rate at which feed is consumed and the efficiency with which it is utilized are important factors to determining growth rate. Estimation of feed consumption in fish is difficult due to lack of accurate methods of evaluation. Gut evacuation time (GET) have been used to estimate feed consumption and utilization in aquaculture.

However, composition of feed ingredients also plays an important role in GET. Bentonite clays are binder used in feed technology to improve physical pellet quality, and capable of increasing feed viscosity. In this study, natural (NB) and acid activated bentonite clay (AB) were include, each at three inclusions (0-control, low -500, High-1500mg/kg) aquafeed's. 120 Fish weighing 240g-240.5g were randomly allocated to treatment diets and fed with known mass of pelletized experimental feed *ad libitum*, 4 fish were selected randomly and carefully dissected to collect stomach content (SC) and intestinal filling (IC) content at (5, 30, 60 and 120mins) post feeding.

Results revealed control diet had significantly ( $p < 0.05$ ) higher values for SC at 5mins, and decreased with time faster, as compared to clay diets. In clay diets, SC at low inclusion decreased ( $p < 0.05$ ) as compared to High inclusion. NB had lower values with time as compared to AB (Table 1). The IC of control diets had higher value ( $p < 0.05$ ) at time 30 and 60mins and decreased ( $p < 0.05$ ) at 120 mins as compared to clay diets. In clay diets, IC at low inclusion had significantly ( $p < 0.05$ ) higher values as compared to High inclusion. IC of fish fed NB diet had higher values as compared to AB (Table 2). This report suggested that dietary bentonite clay affected intestinal filling rate, reduced the rate of feed evacuation in the gut, and may lead to better feed utilization. Acid activated bentonite and high inclusion rate should be used with caution.

Table 1: Effect of natural and acid activated bentonite on stomach emptying rate (SC)

| Time interval         | 0                  | NB                 | AB                  | SEM  |
|-----------------------|--------------------|--------------------|---------------------|------|
| <b>Low Inclusion</b>  |                    |                    |                     |      |
| 5 (mins)              | 14.63 <sup>a</sup> | 13.23 <sup>b</sup> | 11.49 <sup>c</sup>  | 0.01 |
| 30 (mins)             | 7.79 <sup>c</sup>  | 9.68 <sup>b</sup>  | 12.68 <sup>a</sup>  | 0.01 |
| 60 (mins)             | 4.35 <sup>c</sup>  | 6.97 <sup>b</sup>  | 11.10 <sup>a</sup>  | 0.01 |
| 120 (mins)            | 2.91 <sup>c</sup>  | 5.41 <sup>b</sup>  | 9.52 <sup>a</sup>   | 0.01 |
| <b>High Inclusion</b> |                    |                    |                     |      |
| 5 (mins)              | 14.63 <sup>a</sup> | 10.78 <sup>b</sup> | 10.51 <sup>bc</sup> | 0.01 |
| 30 (mins)             | 7.79 <sup>c</sup>  | 10.12 <sup>b</sup> | 10.40 <sup>a</sup>  | 0.01 |
| 60 (mins)             | 4.35 <sup>c</sup>  | 8.76 <sup>b</sup>  | 10.06 <sup>a</sup>  | 0.01 |
| 120 (mins)            | 2.91 <sup>c</sup>  | 6.57 <sup>b</sup>  | 9.78 <sup>a</sup>   | 0.01 |

<sup>abc</sup> means along the same row with different superscripts are significantly ( $P < 0.05$ ) different

Table 2: Effect of natural and acid activated bentonite on Intestinal filling rate (IC)

| Time interval         | 0                 | NB                | AB                 | SEM   |
|-----------------------|-------------------|-------------------|--------------------|-------|
| <b>Low Inclusion</b>  |                   |                   |                    |       |
| 5 (mins)              | 0.00 <sup>a</sup> | 0.00              | 0.00               | 0.001 |
| 30 (mins)             | 0.41 <sup>a</sup> | 0.29 <sup>b</sup> | 0.09 <sup>c</sup>  | 0.001 |
| 60 (mins)             | 0.62 <sup>s</sup> | 0.42 <sup>b</sup> | 0.25 <sup>c</sup>  | 0.001 |
| 120 (mins)            | 0.35 <sup>c</sup> | 0.71 <sup>a</sup> | 0.65 <sup>ab</sup> | 0.001 |
| <b>High Inclusion</b> |                   |                   |                    |       |
| 5 (mins)              | 0.00 <sup>a</sup> | 0.00              | 0.00               | 0.001 |
| 30 (mins)             | 0.41 <sup>a</sup> | 0.20 <sup>b</sup> | 0.04 <sup>c</sup>  | 0.001 |
| 60 (mins)             | 0.62 <sup>s</sup> | 0.32 <sup>b</sup> | 0.11 <sup>c</sup>  | 0.001 |
| 120 (mins)            | 0.35 <sup>c</sup> | 0.46 <sup>a</sup> | 0.52 <sup>ab</sup> | 0.001 |

<sup>abc</sup> means along the same row with different superscripts are significantly ( $P < 0.05$ ) different

## **UTILIZATION OF VEGETABLE WASTE AS A SOLE FEED IN THE DIET OF ROHU *Labeo rohita* FINGERLINGS**

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A study was conducted by using vegetable waste as sole feed in the diet of rohu (*Labeo rohita*) fingerlings. Vegetable waste was comprised of ten different vegetables including Green gourd, Bitter gourd, Ridge gourd, Egg plant, Edible pea, Okra, Potato, Cucumber, Colocasia and Tomato. These vegetable wastes were dried, mixed and analyzed chemically. As the feed was very dry, therefore to make granules 11% gelatin solution was added to it and granules of 2mm diameter were produced. In a trial period of 45 days, three experimental groups and one control group were maintained each containing 12 rohu (*Labeo rohita*) fingerlings. Control group was fed with aquarium feed while experimental groups were fed with vegetable waste. Feeding at 1% body weight, all the groups showed negative growth. When fed at 2% body weight, some fish started gaining weight and when feeding at 2.5% body weight, all the groups started showing positive growth. It was concluded from the present study that vegetable waste cannot be used as sole feed but it can be used as supplementary diet for providing extra protein, fats and vitamin contents.

## ASSESSING CONSERVATION PRIORITIES FOR THREATENED ORNAMENTAL FISHES OF NIGERIA

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About 10% of freshwater fishes of Nigeria are threatened. Of the 31 IUCN Redlisted freshwater fishes in Nigeria, 15 are of ornamental fishes and of importance in aquarium trade. The paper assessed the current status, distribution, life history traits and threats to these at risk ornamental fishes. The endemic Nothobranchid killifishes (7) accounted for most of the threatened species: *Aphyosemion bivittatum*, Vulnerable B1ab(iii)+2ab(iii); *A. bualanum*, Endangered B2ab(iii); *Epiplatys biafranus*, Endangered B1ab(ii,iii,v)+2ab(ii,iii,v); *E. longiventralis*, Vulnerable B1ab(ii,iii,v)+2ab(ii,iii,v); D2; *Fundulopanchax scheeli*, Endangered B1ab(i,ii,iii,v)+2ab(i,ii,iii,v); *F. powelli*, Critically Endangered B1ab(iii)+2ab(iii); D; *F. arnoldi*, Endangered B1ab(ii,iii,v)+2ab(ii,iii,v); Cyprinidae (3): *Barbus sylvaticus*, Endangered B1ab(iii)+2ab(iii); *B. bawkuensis*, Endangered B1ab(iii)+2ab(iii); *Barboides gracilis*, Vulnerable B2ab(iii); Distichodontidae (2), *Neolabias powelli*, Critically Endangered B1ab(i,ii,iii,v)+2ab(i,ii,iii,v); *N. axelrodi*, Endangered B1ab(iii)+2ab(iii); Alestidae (2), the iconic Niger tetras, *Arnoldichthys spilopterus*, Vulnerable B1ab(ii,iii,v)+2ab(ii,iii,v) and *Alestes petrosus mykalai*, Vulnerable A2ce; Anabantidae, *Ctenopoma nebulosum*, Vulnerable B1ab(ii)+2ab(ii). They are all small-sized fishes (<10cm SL), inhabiting marginal aquatic systems like swamps, backwaters, streams and pools, habitats that are easily destroyed by human activities. The study revealed low impact of wild collection, while the most important threat to these fishes is habitat degradation and loss, deforestation and pollution mainly from petroleum industry. The consequences of these threats, suggestions for prioritising conservation efforts and potential management interventions are discussed.

## A REVIEW ON THE EFFECTS OF DIETARY ANTIOXIDANTS ON REDUCING MERCURY TOXICITY IN MARINE FISH AND IN MICE MODELS

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This paper will review on the effects of dietary selenium (Se), vitamin C and E on reducing mercury toxicity in terms of growth and bioaccumulation of mercury in different tissues of juvenile olive flounder, *Paralichthys olivaceus*, as well as in male mice model. A series of experiments were conducted in juvenile olive flounder to evaluate (1) the effects of Se alone (2) the effects of vitamin C alone (3) the effects of vitamin E alone (4) synergistic effects of Se and vitamin C (5) synergistic effects of Se and vitamin E (6) synergistic effects of vitamin C and E (7) synergistic effect of Se with vitamin C and E, on induced mercury toxicity (20 mg/kg diet) in juvenile olive flounder. First experiment, findings indicated that fish fed Se at 2 mg/kg diet showed growth improvement and reduced mercury concentration in muscle tissue of juvenile olive flounder. Second experiment, dietary 200 mg vitamin C/kg diet have detoxification effects on mercury toxicity in fish. Third experiment, dietary 200 mg vitamin E/kg diet have detoxification effects on mercury toxicity in fish. Fourth experiment, dietary 400 mg vitamin C/kg diet with 4 mg selenium/kg supplemented diet had synergistic detoxification effects on mercury toxicity in fish. Fifth experiment, dietary vitamin E more than 100 mg/kg diet with 2 or 4 mg Se/kg-supplemented diet had synergistic detoxification effects on mercury toxicity in juvenile olive flounder. Sixth experiment, the results showed that dietary 400 mg vitamin C with 200 mg vitamin E/kg diet had synergistic positive effect on mercury toxicity. Seventh experiment, the best combinations of the dietary antioxidant levels could be  $C_{800}E_{100}Se_2$  and  $C_{800}E_{200}Se_2$ /kg diet for mercury detoxification in juvenile olive flounder. In mice model, the results revealed that male ICR albino mice fed dietary Se, vitamin C and E at 2, 400 and 200 mg/kg diet, respectively, showed synergistic detoxification effects on induced mercury toxicity.

## **STRENGTHENING THE EFFECTIVENESS OF AQUACULTURE AND FISHERIES SCIENCE CURRICULA IN AFRICA**

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Fisheries and aquaculture development and management in Sub-Sahara Africa (SSA) have been practiced for almost a century, and yet, the sector productivity continues to lag behind other forms of agriculture (crop and livestock). It is also noted that just as with crop and livestock sciences, there are well established curricula for fisheries and aquaculture at various levels but the limited effectiveness of the curricula may partly explain why fisheries and aquaculture practices are not at similar levels with those in other forms of agriculture. Despite progress made in promoting and mainstreaming fisheries and aquaculture disciplines into governance structures, wild capture fisheries in SSA have either stagnated or have declined primarily due to unsustainable, uncontrolled fishing. In the case of aquaculture, it may be reasoned that this sub-sector is not a traditional agricultural practice. However, this argument cannot be sustained considering other more successful but non-traditional practices such as the coffee, cocoa, tea and poultry industries. Decades of investments by governments and donor support in development of aquaculture would be expected to have stimulated the sub-sector to levels comparable at least to Egypt, the leading producer of aquaculture products in Africa with volumes averaging 800,000 tonnes annually.

Fisheries, aquaculture and related industries have the potential to support more people in SSA with likely further increases in aquaculture. The harvest from capture fisheries, sale and processing of fish contribute directly to food and employment by increasing incomes at household, national and regional levels. Some dimensions in the global economy related to fish (e.g. the role of fish in human development at key life stages, physical and mental development and health) add to the significance of fisheries and aquaculture. Therefore, the need for capacity development in the two sub-sectors cannot be ignored. It is essential to find out the critical needs in the science curricula which if addressed could make an effective contribution to the fish value chain. Whereas the existing curricula generally address the objectives of learning, we examine major constraints in the fish value chain that could be incorporated into curricula with the aim of producing more practically oriented graduates and a competitive sustainably utilized sector.



## METABOLIC RESPONSE TO FEEDING RATES IN JUVENILE BRAZILIAN SARDINE *Sardinella brasiliensis*

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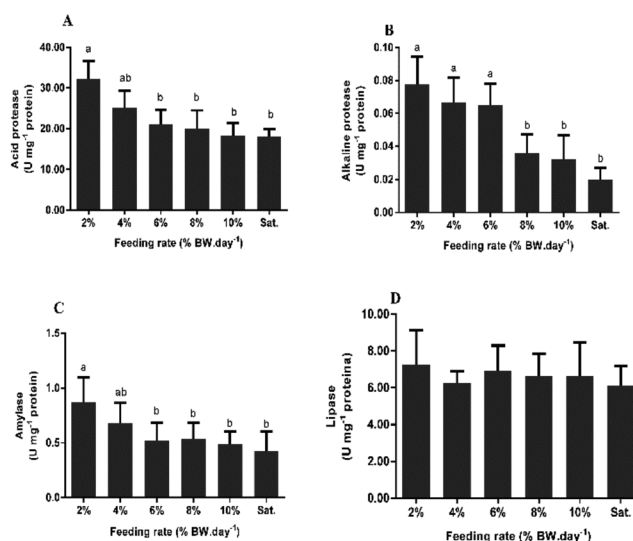
Brazilian sardine is one of the most commercially important fishery resources caught along the Southeastern Brazilian Bight. It is currently captured by various fleets for use in the canning industry and extensively as live bait for skipjack tuna fishery, and its availability varies seasonally due to over-fishing and environmental changes. Commercial catches began in the late 1950s, and had quick growth in the 1960s, reaching a peak of 228000 tons in 1973. However, in 2011, production was only 75 223 tons. Considering its economic importance, one of the efforts that could avoid collapse of the Brazilian sardine is its production in captivity. However, to evaluate and develop optimal conditions for the production of new aquaculture species, among other requirements, it is necessary to establish an appropriate feeding protocol. Few studies have considered the potential effects of feeding rate on the specific activity of digestive enzymes and plasma metabolites. Studies of digestive enzymes in fish could help to develop nutritional strategies for fish feeding procedures and diet formulation. In addition, the utilization of dietary nutrients can be reflected in the metabolic profile in various fish tissues. The present study was conducted to evaluate the effects of feeding rate on plasma metabolites and digestive enzyme activities in Brazilian sardine juveniles.

Juveniles weighing about  $1.69 \pm 0.26$  g were fed on a commercial diet for 41 days at various daily feeding rates of 2, 4, 6, 8, 10% BWday<sup>-1</sup> (body weight per day) and 10.3 (satiation), and the plasma metabolites and digestive enzyme activities were determined. The increased feeding rates significantly affected total proteins, cholesterol and triglycerides, while glucose levels scarcely changed (Table 1).

Acid and alkaline proteases and amylase activities were significantly decreased with increasing feeding rates, and there were strong positive correlations between enzyme activities and feeding rates, while lipase activity remained relatively constant (Figure 1). The results suggest that low feeding rates stimulate mobilization of metabolites to supply the energy needed to maintain overall metabolism, while digestive enzyme activities increase to enhance feed utilization.

| Feeding Rate<br>(% BW day <sup>-1</sup> ) | TP<br>(g L <sup>-1</sup> ) | GL<br>(mmol L <sup>-1</sup> ) | CH<br>(mmol L <sup>-1</sup> ) | TG<br>(mmol L <sup>-1</sup> ) |
|---|----------------------------|-------------------------------|-------------------------------|-------------------------------|
| 2   | 12.17 ± 3.04 <sup>c</sup>  | 3.49 ± 0.90                   | 0.96 ± 0.28 <sup>d</sup>      | 2.31 ± 0.35 <sup>d</sup>      |
| 4   | 18.08 ± 2.86 <sup>bc</sup> | 3.91 ± 0.64                   | 1.74 ± 0.33 <sup>cd</sup>     | 3.07 ± 0.65 <sup>d</sup>      |
| 6   | 20.39 ± 3.95 <sup>b</sup>  | 4.78 ± 0.50                   | 2.32 ± 0.39 <sup>bc</sup>     | 5.01 ± 0.52 <sup>c</sup>      |
| 8   | 20.33 ± 4.14 <sup>bc</sup> | 5.13 ± 1.27                   | 2.87 ± 0.49 <sup>b</sup>      | 5.64 ± 0.58 <sup>cb</sup>     |
| 10  | 22.58 ± 4.07 <sup>b</sup>  | 4.95 ± 1.87                   | 2.93 ± 0.33 <sup>b</sup>      | 6.40 ± 0.48 <sup>ab</sup>     |
| Satiation                                 | 29.40 ± 2.56 <sup>a</sup>  | 5.07 ± 1.25                   | 3.68 ± 0.29 <sup>a</sup>      | 6.62 ± 0.51 <sup>a</sup>      |

TP: Total proteins; GL: glucose; CH: cholesterol; TG: triglycerides. Values in the same column with different letters are significantly different ( $P < 0.05$ ).



## MARINE RESOURCES FOR A HEALTHIER FUTURE: MEETING THE ENVIRONMENTAL, ECONOMIC, AND TECHNOLOGICAL CHALLENGES OF THE OCEANS

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The pace of technological change in recent decades has opened new horizons for the deeper understanding and better utilization of the marine resources. On the other hand, there are emerging challenges, which have to be tackled, such as the overexploitation of wild marine resources, the pollution of the hydrosphere, and the growing health concerns and awareness of consumers and its impact on a greater emphasis on seafood quality. The gist of the DivAV/IPMA mission is precisely to find adequate and viable answers to these problems using state-of-the-art technology. Indeed, this department has been actively working in overcoming hurdles essential for meeting the aforementioned challenges and to provide new technological solutions. DivAV/IPMA is excellently poised to both transform material resources into knowledge (Research) and, in turn, translate this knowledge into added value with benefits for all marine sector stakeholders (Innovation). This is possible on the basis of extensive expertise and experience in seafood science and technology, in extracting valuable fractions from resources, preparing novel functional products, and in analysing marine-based products for a thorough quality assessment. Specifically, this department seeks to explore the potential of meaningful marine resources that are undervalued —from seaweeds to underutilized fish species and to by-products of main commercial fish species processing— through the combination of novel processing technologies and innovative approaches. This effort should help the environment in that the intensity of exploitation of some resources is lowered and balanced by intelligent upgrading of other biomass sources. Of course, this endeavour needs to be followed by a stringent focus on the conception, development, and analysis of new seafood, new feeds for farmed fish, and novel biotechnological products as well as a permanent and interactive connection to industry partners, helping them in testing technologies and products at an industrial scale. DivAV/IPMA has achieved large successes in several key areas, namely, it has advanced in aquaculture (emergent species and higher fish health and quality), seafood quality (wide array of methodologies from physical to biochemical, textural, and organoleptic), marine bioactive prospection and isolation, seafood lipidomics (species profiling), determination of bioaccessibility (*in vitro* models of human digestive system), and risk-benefit assessment. Therefore, it has been and strives to be in the future an agent promoting scientific progress, creating and testing new technological solutions, disseminating knowledge, and enhancing consumer health and environmental awareness.

In this oral communication, will be presented two different case studies concerning the upgrading and processing steps of a new aquaculture species as meagre and innovative adding value techniques for the macroalgae species based in quality parameters, focusing the application in new food products.

**Acknowledgments:** Research funded by project I9+ProAlga (MAR2020, Portugal)

# DEVELOPING DISPERSAL MODELLING TOOLS TO PREDICT REGIONAL ENVIRONMENTAL IMPACTS OF LARGE SCALE FIN-FISH AQUACULTURE

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The ability to achieve environmentally sustainable Atlantic salmon aquaculture in coastal ecosystems is presently constrained by our limited knowledge of the regional interactions and fate of organic effluents on benthic ecosystems. A first step in addressing this limitation is to establish verified predictive modelling tools to better understand local and regional dispersion of organic effluents in the wider ecosystem.

By combining settling velocity of faecal waste from different sizes of Atlantic salmon, a 3D hydrodynamic model coupled with a particle tracking model and farm management data, this presentation will demonstrate the accuracy and reliability of a generic far- and near-field effluent dispersion model to predict the spread of particulate organic waste into fjord ecosystems. Simulations predict that more than 75% of organic effluents are dispersed to near-field sites (< 500 m from the release point), while a small proportion of particulate organic effluents (up to 2.7%) are dispersed to far-field sites (> 2 km) (Figure 1).

Modelling dispersion of organic material dispersed from fin-fish farms in a whole fjord system (i.e. 50 000 tons of organic loading per year), demonstrates that organic effluents from fish farms may be spread over large areas of fjord systems with the potential for overlapping between multiple fish farms, potentially leading to hotspots of organic accumulation. This modelling tool has the potential to be used as a premise for selecting optimal sites for monitoring regional benthic impacts of fin-fish aquaculture.

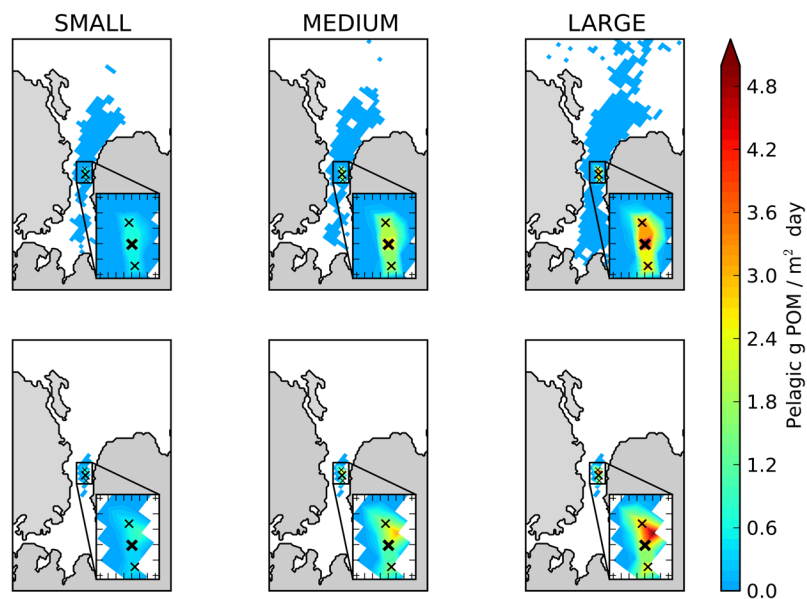
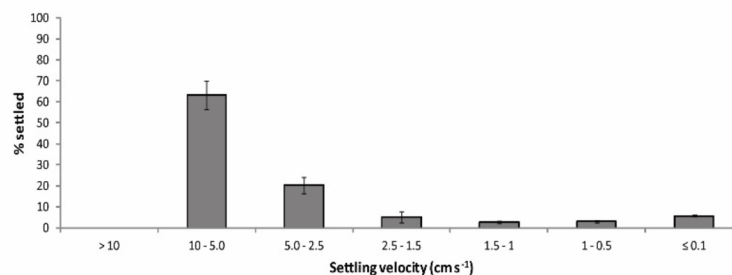


Figure 1: Multiple spreading simulations of organic effluents released from net pens containing Atlantic salmon of three different sized fish (Small - 0.6 kg; Medium - 1.5 kg; and Large - 3.5 kg) in a Norwegian Fjord.



## DIETARY INCLUSIONS OF DRIED MACROALGAE MEAL IN FORMULATED DIETS IMPROVE THE GROWTH OF GREENLIP ABALONE *Haliotis laevis*

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Wild greenlip abalone predominantly consumes macroalgae, but under culture conditions in Australia are fed formulated diets. Dried macroalgae meals are promising ingredients for abalone diets. In this 92 day study, the growth, feed utilisation and digestive enzyme activities of greenlip abalone (*Haliotis laevis*; 2.89 g) fed dried macroalgae meals (*Ulva* sp. meal [supplied by Venus Shell Systems, Narrawallee, NSW, Australia]. or *Gracilaria cliftonii* meal [referred to as *Gracilaria* sp. Meal; produced at South Australian South Australia Research and Development Institute South Australian Aquatic Sciences, West Beach, SA, Australia]) in formulated diets were investigated. Seven experimental formulated diets were used, a basal diet (0 % macroalgal diet), and three inclusion levels of *Ulva* sp. meal (5, 10 and 20 % inclusions) and *Gracilaria* sp. meal (5, 10 and 20 % inclusions). Diets were formulated to contain 35 % crude protein, 5 % crude lipid and 17.5 MJ kg<sup>-1</sup> gross energy. An Australian commercial diet was also fed to abalone and compared to the 0 % diet. Growth and feed conversion ratio (FCR) of abalone fed the 0 % diet and commercial diet were similar. Abalone fed 5 % *Gracilaria* sp. meal or *Ulva* sp. meal exhibited superior growth to abalone fed 0 %. However, increasing dietary *Gracilaria* sp. meal inclusions (>10 %) led to further growth improvements, but impaired protein and energy retentions. In contrast, abalone fed >10 % *Ulva* sp. meal inclusions exhibited similar growth to those fed 0 and 5 % *Ulva* sp. Although *Ulva* sp. and *Gracilaria* sp. meals are currently not commercially viable, this study clearly demonstrates the potential to develop abalone feeds with inclusions of dried macroalgae meal. We recommend a dietary inclusion of 10 % *Gracilaria* sp. meal or 5 % *Ulva* sp. meal to improve abalone growth.

## INTERMITTENT FEED-INDUCED HYPOXIA EFFECTS THE GROWTH AND FEED UTILISATION OF LARGE YELLOWTAIL KINGFISH *Seriola lalandi* AT SUMMER WATER TEMPERATURES

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Hypoxic conditions during and post-feeding may be problematic for the Yellowtail Kingfish (*Seriola lalandi*) aquaculture industry, particularly during low tidal water movement. In this 85 day study, the effect of dissolved oxygen saturation level and intermitted feed-induced hypoxia during and post-feeding on the growth, was investigated on feed utilisation and health of large Yellowtail Kingfish (2.15 kg). Fish were exposed to four dissolved oxygen saturation treatments, (1) 100% constant, (2) 85% constant; (3) 85% constant with a 3 h hypoxic event (60%) implemented daily post-feeding to simulate a daily feed induced-hypoxic event; and (4) 85% constant with a 3 h hypoxic event (60%) post-feeding implemented twice fortnightly (day 13 and 14 of each fortnight) to simulate feed induced-hypoxic event during twice fortnightly periods of low tidal flow (dodge/neap tide). Fish were fed a commercial extruded diet to apparent satiation once daily at 9:00 h.

Yellowtail Kingfish exposed to intermittent feed induced-hypoxia (Treatment 4) exhibited significantly reduced specific growth rate (SGR) and feed conversion ratio (FCR), compared to fish exposed at other oxygen treatments investigated in the current study. Yellowtail Kingfish held at constant 100% and 85% dissolved oxygen saturation constant, and fish held at 85% and dropped to 60% for 3 h daily exhibited similar SGR and FCR. However, there was a tendency for SGR to decrease for fish held at a constant 100% to a constant 85% to 85% dropped to 60% for 3 h daily. This study demonstrates that Yellowtail Kingfish may be able to adapt to consistent conditions, including a daily hypoxic event, while fish exposed to intermittent hypoxic events exhibit inferior growth and feed utilisation. In terms of feed management, commercial producers of Yellowtail Kingfish may be able to mitigate exposure to intermitted feed-induced hypoxic events by selecting sites with adequate water flow or by utilising nets with larger mesh sizes to allow greater water exchange. Further research in pilot scale commercial trials are needed to validate these potential feed management solutions.

## SKELETAL DEFORMITIES AT IPMA'S AQUACULTURE RESEARCH STATION (PORTUGAL)

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Fish with skeletal deformities are a serious problem in the aquaculture industry and can affect till 30% of production. Malformations can negatively affect growth rates and the commercial value of the produced fish. Therefore, it is important to identify these abnormalities as soon as possible to discarded fish and be able to avoid increasing production costs.

Aquaculture Research Station (EPPO) is a national facility where research projects are developed to provide answers to aquaculture sector. At EPPO, skeletal deformities are assessed on several Mediterranean fish species: European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), zebra seabream (*Diplodus cervinus*), white seabream (*Diplodus sargus*), common two-banded seabream (*Diplodus vulgaris*), sole (*Solea senegalensis*) and meagre (*Argyrosomus regius*). During rearing, visual observation are firstly used to identify malformation occurrence, a common procedure in aquaculture facilities. Secondly to refine this selection soft X-ray is used to confirm and characterize skeletal deformities on digital images.

Lordosis and vertebral fusions are the main malformations observed in *D. labrax*, *S. aurata*, *D. sargus* and *D. vulgaris* revealed a high incidence of lordosis and kyphosis.

Lordosis, kyphosis, vertebral fusions, mandibular deformities and saddleback-like syndrome were the most common skeletal deformities observed.



**Acknowledgments:** Research funded by project DIVERSIAQUA (MAR2020, Portugal).



# **OCCURRENCE OF TRICHODINID PARASITES OF WILD AND CULTURED *Oreochromis niloticus* AND *Coptodon rendalli* IN LAKE KARIBA, ZIMBABWE**

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This presentation reports the occurrence of trichodinid ciliate parasites on two cichlid species *Oreochromis niloticus* and *Coptodon rendalli* in Lake Kariba, Zimbabwe. Both species were sampled from the wild at three sites using beach seine netting, whereas only cultured *O. niloticus* was sampled from a local fish farm in the Sanyati Basin of the lake. Air dried smears from the body and gills of the fish were prepared and impregnated with 2% aqueous silver nitrate and examined for trichodinids. Morphometric measurements of the trichodinids were carried out and these were used for identification of the species. Counts of the trichodinids on the slides were done to estimate the parasite abundances. Five new Zimbabwean records of trichodinid species were found, viz *Trichodina heterodentata*, *Trichodina centrostrigeata*, *Trichodina compacta*, *Trichodina microspina*. The compound community of all the trichodinids displayed a typical parasite aggregation distribution. The majority of the trichodinids were located on the gills of both host fish. The trichodinid species were not host specific as they were all obtained from both host species. *Oreochromis niloticus* had significantly higher parasite abundances than *C. rendalli* (Mann-Whitney,  $P < 0.01$ ). All wild *O. niloticus* sampled in the study had parasite abundances that were not significantly different (Kruskal-Wallis,  $P > 0.50$ ). When all the *O. niloticus* populations including the farmed fish were compared the abundances were found to be significantly different (Kruskal-Wallis,  $P < 0.01$ ). A similar site comparison for *C. rendalli* was not carried out since it occurred at only two sites and in very small sample sizes. The condition factors of both host fish were not significantly correlated to the abundance of the trichodinids in *O. niloticus* (Spearman Rank,  $P > 0.05$ ) and *C. rendalli* (Spearman Rank,  $P > 0.05$ ). We concluded that more extensive research extending to more host species and a wider sample area was required. There is need for histopathological assessments to establish the impact of the parasites on host tissue, and use of molecular techniques is recommended to ensure accuracy in parasite identification. Finally, more studies on both wild and aquaculture systems are needed to improve productivity and bio-security.



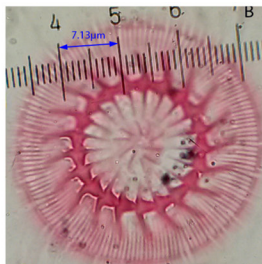
*Oreochromis niloticus*



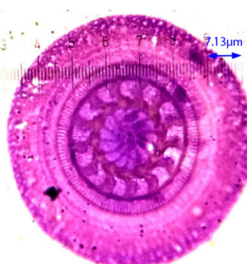
*Coptodon rendalli*



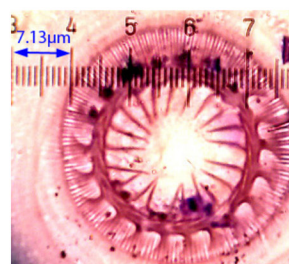
*T. heterodentata*



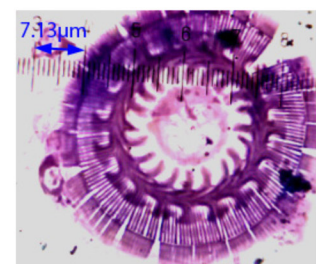
*T. centrostrigeata*



*T. compacta*



*T. microspina*



*T. acuta*



## **OXIDATIVE STRESS IN GILLS AND DIGESTIVE TRACTS OF PACIFIC OYSTER, *Crassostrea gigas*, AND JAPANESE SCALLOP, *Patinopecten yessoensis*, CAUSED BY *Dinophysis caudata***

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Diarrheic Shellfish Poisoning (DSP) is one the recognized symptom types of shellfish poisoning. The poisoning is caused by the consumption of shellfish, typically bivalve molluscs, that contain okadaic acid (OA) and/or its derivatives (DTX). OA and DTX, and other lipophilic toxins the pectenotoxins (PTX), are produced mainly by species of the cosmopolitan dinoflagellate genera, *Dinophysis*. Bivalve molluscs, following filter-feeding on *Dinophysis* cells, accumulate the toxins. The possible effect(s) of these toxins on the shellfish has not been well investigated to date.

Under laboratory conditions, adult Pacific oysters, *Crassostrea gigas*, and Japanese oysters, *Patinopecten yessoensis*, were fed cultures of *Dinophysis caudata* for one week, at different daily rations. At the end of the feeding experiments, the gills and digestive tracts were immediately dissected and preserved at – 80° C until analyses.

The activities of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), glutathione-*S*-transferase (GST), and glutathione peroxidase (GPx) were assessed based on colorimetric assays, and measured spectrophotometrically. The up-regulation and down-regulation of these oxidative enzymes in the gills and digestive tracts of oysters and scallops are presented, and the biological implications of these regulations in the effects of *D. caudata* in oysters and scallops are discussed.

## AMMONIA EXCRETION AND NITROGEN ESTIMATION DURING FEEDING IN *Octopus cf. vulgaris*

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The cosmopolitan *Octopus cf. vulgaris* is a potential species to diversifying aquaculture due to their zootechnical potential and high market value. However, there is no information with direct applications to commercial aquaculture for the Brazilian *O. vulgaris* since is being redescribed as a new species of “vulgaris complex” (*in elaboration*). We estimate the ammonia excretion and nitrogen produced during octopuses feeding in laboratory as a useful tool for improving the management of future installations of octopus on-growing and in an attempt to minimize environmental impacts.

To do this, 14 males of *O. vulgaris* were caught by fishery in the Florianópolis Island, Southern Brazil and transported to the laboratory facilities. The experiment carried out in 3 cylindrical indoor tanks (4 m diameter) in open flow-through seawater system (6 L.h<sup>-1</sup>) with constant aeration. Following one-week acclimatization period, the males with initial weight=1.5±0.6 kg were randomly distributed into the tanks. The experimental period lasted four days under 10:14 h light–dark photoperiod. Water temperature, salinity, OD and pH (mean ± SD), measured once a day were 19.9±3.3°C, 33±0.2 g.L<sup>-1</sup>, 6.6±0.3 mg.L<sup>-1</sup> and 8.0±0.2, respectively. Octopuses were fed once a day (10:00 am) with fillets de-frozen (excluding viscera, head and bones) of a low market value Atlantic bigeye *Priacanthus arenatus*, at 5% of biomass. The uneaten food was cleaned on a daily (13:00 am) and dried before being weighed. The ingestion rate was obtained by the difference between food provided and uneaten portion. Samples of water were collected before, during and after octopuses feeding to evaluate total ammonia-nitrogen (NH<sub>3</sub>+NH<sub>4</sub><sup>+</sup>), nitrite and nitrate. The total nitrogen (TN) was estimated by the formula: TN (mgN tank<sup>-1</sup>.h<sup>-1</sup>) = [N (max) – N(min)] x F, where: N (max)= nitrogen maximum (mg.L<sup>-1</sup>), nitrogen minimum (mg.L<sup>-1</sup>), flow (L.h<sup>-1</sup>).

The ingestion rate was 26.18 g.kg<sup>-1</sup>, which correspond to 93% of the diet uneaten. Thus, octopuses well not accepted the diet. To feed octopuses with Atlantic bigeye during four days, were produced 3,45 mgN.tank<sup>-1</sup>.h<sup>-1</sup> which correspond to the amount excreted by the octopus, lixiviation of food uneaten and the quantity produced by bacteria and fungi present in the water.

**Table 1. Concentrations (mg.L<sup>-1</sup>) of total ammonia nitrogen and nitrite measured before, during and after feeding in *O vulgaris*.**

|                | Before     | During     | 2h after  | 6h after  |
|----------------|------------|------------|-----------|-----------|
| <b>TAN</b>     | 0,41±0,17  | 0,48±0,15  | 0,51±0,20 | 0,47±0,13 |
| <b>Nitrite</b> | 0,01± 0,01 | 0,02± 0,01 | 0,02±0,01 | 0,03±0,01 |
| <b>Nitrate</b> | 1,63± 0,60 | 1,81±0,78  | 2,02±0,87 | 2,25±1,05 |

## EFFECTS OF DIFFERENT PROTEIN AND LIPID RATIOS ON GROWTH, FISH QUALITY AND HEAVY METALS BIOACCUMULATION DURING A COMPLETE GROWOUT CYCLE OF RED DRUM *Sciaenops ocellatus* IN A RECIRCULATED AQUACULTURE SYSTEM

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Tropical fish are generally growing much faster than temperate species and a harvest size of over 1.5 kg can be obtained in 18 months. Therefore, the optimal composition of diets to support these high growth rates is likely to be very different from traditional marine fish feeds used for temperate species. Moreover, little is known about the effects of different feeds on the quality and health of the large fish harvested. A few nutritional studies have been undertaken on red drum *Sciaenops ocellatus* in order to evaluate the dietary requirements of the species and its tolerance to terrestrial feedstuffs as replacement of fishmeal-based proteins. But, most published studies have been done on small-size fish over a short period of time and thus did not cover the whole grow-out period. The objectives of this two year experiment were to determine optimal digestible protein/ digestible energy ratios for growth, FCR and fish quality of red drum juveniles and adults up to market size. Three diets were formulated with the same raw materials and similar composition except for the %protein and %lipid levels, which varied from 48/12 (diet A) to 48/15.5 (diet B) and 44/15.5 (diet C).

During the first phase of the experiment fish were grown from 30g to plate size (300g) in closed recirculation systems. Juvenile red drum originating from the same spawning event from domesticated brood-stocks were randomly distributed to 9 tanks of 104 fish each (3 replicates per test diet). Feeding was done continuously and at maximal feeding rate for all treatments.

After 102 days of culture, the fish raised on diet A reached 262 grams in average, versus 248 grams for diet B and 226 grams for diet C. Diet A outperformed the other diets in terms of growth with average SGR of 4.51% for diet A, 4.45% for diet B and 3.83% for diet C. FCRs were 1.26 for A, 1.34 for B and 1.55 for C. For the second phase of the experiment, 36 fish from each treatment of the first phase were pooled and grown in closed recirculation systems until fish from the fastest-growing treatment reached filet size (2,2kg).

After 542 days of culture, fish raised on diet A reached 2265 grams, versus 2025 grams for diet B and 1263 grams for diet C. Average SGRs during the second phase were 0.59% for diet A, 0.63% for diet B and 0.60% for diet C. FCRs were 1.58 for A, 1.42 for B and 1.68 for C.

Additionally, liver condition and hepatosomatic index were evaluated per treatment and the bio-accumulation of 5 heavy metals (lead, cadmium, arsenic, mercury, and fluor) were monitored in the liver and fillets during the feeding experiment. Finally, the fillets from the different treatments were subjected to taste panels for evaluation of the sensory characteristics by taste panels. Fillets from fish fed with diet A obtained the best scores and were most appreciated in the organoleptic evaluation by both naive consumers and trained panels. Generally, low heavy metal bioaccumulation was observed in both flesh and liver with values often under the limit of detection.

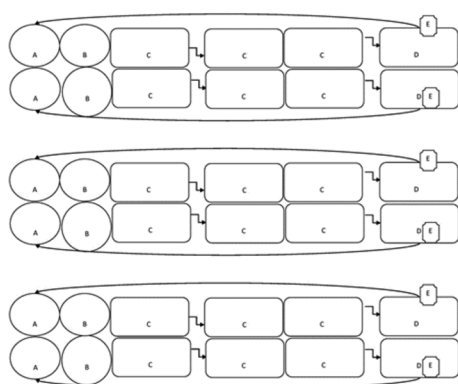
## ARID LAND AQUAPONIC SYSTEM OF TILAPIA *Oreochromis spilurus* AND Quinoa *quinoa* under two different salinities; PRODUCTION CHARACTERISTICS, IN INPUT AND OUTPUT

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Aquaponic especially in Arid Zones would be the healthiest and most efficient food production system. It uses less than 90 percent of water used in convention crop production. Six small aquaponics raft similar to University of Version Island UVI system experiment was set to test tilapia *Oreochromis spilurus* fingerlings growth with quinoa *Chenopodium quinoa* at two salinities levels 0 and 4ppt in triplicate. Each unit consists of one fish tank followed by a settling and biological filter tank in one then three half tanks covered with floated perforated Styrofoam where quinoa seeds were inserted in a piece one-inch Rockwool cube inside some perforated bottom plastic cubs. Sludge was removed from each system separately. Water flow rate was 0.310 m<sup>3</sup> hour<sup>-1</sup>. Fish tanks were stocked at a rate of 100 fish m<sup>3</sup> with an average weight of 5 g. tilapia the experiment lasted for 105 days. Fish were fed daily three times a day to satiation of commercial floating tilapia feed. Quinoa seeds were inserted in a piece one-inch Rockwool cube inside some perforated bottom plastic cubs. Raceways were planted in perforated Styrofoam at a rate of 24 quinoa *Chenopodium quinoa* per square meter. Quinoa *Chenopodium quinoa* growth was slightly less in higher salinity aquaponics units as compared with the low salinity water one. On the other hand, quinoa seeds production per plant were similar production. per plant was slightly less There was no difference between treatments in fish growth parameters namely weight gain, feed intake, feed conversion rates and protein sufficiency ratios. Fish weight reached 130 g fish<sup>-1</sup> quinoa produced 35 g of seeds per plant during the experimental duration. Water quality were monitored as followed; DO 7.3 to 8.1, pH 6.3 to 7.9, NH<sub>3</sub> 0.03 to 0.52 mg per liter, NO<sub>3</sub> 7.3 to 8.6 mg per liter.



Layout of the Aquaponic units

A - FISH STOCKING TANK B – BIO FILTER TANK

C – PLANT CULTIVATION RACE WAY

D – WATER COLLECTING AND PUMPING TANK

E – ELECTRICAL SUMP

## **BUILD MORE BRIDGES BETWEEN AFRICA & ASIA FOR SUSTAINABLE AQUACULTURE**

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Fish is the best and largest source of animal protein in the world. Over 130 million metric ton (mmt) of seafood per year is consumed as compared to 114, 106, and 68 mmt of pork, chicken and beef respectively. The world per capita fish consumption is about 20 kg per year. Asia has more than that whereas Sub-Saharan Africa has less than half of the world average. Fish consumption in most African countries declined due to decline in wild catch when demand for seafood is rising globally. Over 9 billion people expected to reach in the world by 2050 will need double amount of fish from aquaculture from its current production of 75 mmt/year assuming wild catch remains constant. Aquaculture has been considered the only way to achieve sustainable seafood and nutrition security. In the context of climate change, farming of fish has been emphasized as it produces 2-7 times less green house gases and converts feed to meat 2-6 times more efficiently than chicken, pig and cattle.

Most developed countries tried to aquaculture during 1970-80s but they couldn't sustain. They started supporting developing nations to promote aquaculture so that they could import. Fish farming received more attention in Asia where people were farming fish traditionally. Asia produces about 90% of the total farmed fish and has 8 out of 10 top fish producing countries. Among the aquaculture species in Asia, shrimp ranks highest in terms of value and trade and over 90% is produced in Asia. However, it requires coastal saline water for its farming. Many African countries with huge coastal line could try adopting very attractive business. In terms of volumes, farming of carps is far ahead of others. Indian and Chinese carps are farmed utilizing planktons as natural food often enhanced by application of organic and/or inorganic fertilizers. This traditional method has tremendous contribution to livelihood of millions of people especially in rural areas.

Remarkable success has been achieved in farming of tilapia which originally came from Africa, and is now spreading to about 150 countries and total annual production has reached over 5 mmt becoming the second largest group after carps. Millions of farmers in Bangladesh, Laos, Thailand, the Philippines, Vietnam and other countries are earning well and taking nutritional benefits from tilapia. Another success story is about Pangasius. In Vietnam, its farming took off mainly due to export potential. In a decade's time, annual production reached over one mmt; over 90% of which is exported worldwide. About 40,000 farmers do farming, and over 0.2 million people work in processing and marketing businesses. Bangladesh, India, Indonesia and other countries are also growing Pangasius learning from Vietnam.

Asia is producing not only the species seen as successful but also a wide variety of aquatic species including amphibians are tried to explore new species for culture. By trial and error, some farmers become very specialized for some species, or certain life stages such as breeding, nursing or grow-out of particular species e.g. catfish, snakehead, marble goby, gourami etc. There are unbelievable records in Asia. For examples, sales of 20 million monosex tilapia fry per month, 5 million catfish hatchlings per day, 4-5 tons of tilapia or snakehead fish sales per day and so on by a single farm mostly managed by family members, often headed by a woman. These successful commercial farmers are generating over US\$10,000 revenue per day. There are many other unwritten other success stories. To know and learn these, interested individuals or groups have to visit their fields and have to observe their day-to-day operations as a part of hands-on training or study tour. However, access to real information, shortages of technologies, and prohibitively high cost of trip from Africa to Asia have been major constraints. Some attempts have been made in assisting professionals to go to Asia e.g. Cote D'Ivoire, Kenya, Nigeria, Tanzania, Zambia and so on but these are not adequate. Therefore, as a part of south-south cooperation, more bridges, not the walls, are needed between Africa and Asia to develop sustainable aquaculture in Africa, which has a huge potential to feed its people and the world.

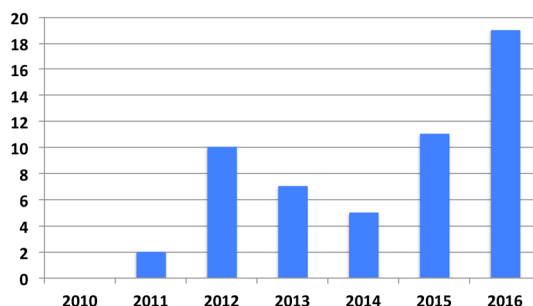
## ROLE OF EDUCATION, RESEARCH AND TRAINING IN ASIAN AQUACULTURE DEVELOPMENT

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In many parts of Asia, fish were abundant in rivers, lakes, swamps and rice fields. People living in the flood plains grow rice as a source of energy, and catch aquatic animals as a good source of protein. Therefore, Asian societies especially in South East Asia are often called as rice-fish societies. Farming of fish or aquatic animals might have begun either confining fish in rice plots which entered during rainy season or holding of surplus live fish caught from the wild in inverted mosquito nets to consume later. However, systematic research and development of farming of aquatic animals and plants defined as aquaculture began only since 1950s. Universities used to offer mostly basic fisheries courses either under zoology or other similar faculties or departments. Not many universities had aquaculture as a separate unit or department. AIT started offering a specialized aquaculture program in 1981, and has already trained over 500 lecturers, government officers, NGOs staff and private managers who are holding high positions and playing key roles in aquaculture development through education, research and training in their respective countries. It has further helped directly or indirectly to accelerate the pace of aquaculture development in the region. Collective approach of aquaculture education, research and training has been towards transforming subsistence farming to commercial farming for the benefits of wider communities with the improvement in the system's productivity and profitability.

AIT has been constantly moving ahead carrying out new research and developing new academic degree curricula as well as training courses with the supports from DANIDA, DFID, EU, SIDA, USAID and several others. Some of its partner universities that had only Bachelor's degree in aquaculture have now MSc and PhD programs very successfully running. Similarly, considering the need of specific skills with the changing contexts, short term courses are also revised frequently especially making need-based. Older trainings offered during 1990s were 1-3 months long. The new ones have only 1-3 weeks and were offered during 2010-2016. It attracted a total of 448 professionals from public as well as private sector from all the continents. In terms of number of participants, Africa is second to Asia, however only 12% (54) of the total attended the training courses. Nevertheless, the number is increasing annually (Fig 1). Most of them were for tilapia hatchery and grow-out farming. More participation is expected from Africa as the industry is gradually growing especially in Ivory Coast, Ghana, Kenya, Uganda, Zambia, Zimbabwe and so on. In the past, mostly government officers supported by public funding were attending the training. The new courses are attracting increasingly more from private sector. It is an indication that aquaculture has become a competitive and profitable business, and showing a sign of becoming mature industry.



**Fig. 1** Number of participants from Africa trained by AIT Aqua-Centre

## THE POTENTIAL INFLUENCE OF GENOTYPE X ENVIRONMENT INTERACTION ON THE PERFORMANCE OF FOUR NILE TILAPIA (*Oreochromis niloticus* L.) STRAINS UNDER DIFFERENT NUTRITIONAL REGIMES

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Four Nile tilapia (*O. niloticus*) strains were subjected to two nutritional regimes to quantify potential genotype by environment (GxE) interaction and the effect thereof on the performance of the strains. Nutritional treatments comprised of a high-energy (HE) diet consisting of a commercial tilapia feed, and a low energy (LE) diet consisting of duckweed (*Lemna minor*). The four strains received the treatment diets during a 70-day growth period. Body weight (BW), standard length (SL) and total length (TL) were recorded fortnightly, and were used to calculate specific growth rate (SGR), percentage weight gain (PWG), mean weight gain (MWG), average daily weight gain (ADWG), average daily length gain (ADLG) and average daily standard length gain (ADSLG), condition factor (K), and yield. Biserial correlations were used to quantify the potential influence of GxE interaction on body weight, standard length and total length. Overall, strains that received the HE diet had improved growth rates, when compared to the LE treatment groups. The GIFT strain (S4) performed significantly better than other strains (S1, S2 and S3) in relation to all parameters in the HE treatment groups. Strains on the LE diet did not differ in terms of the abovementioned parameters. Strains subjected to the HE and LE diets did not differ in terms of survival rate. Phenotypic correlations for the strains ranged from 0.906 to 0.952 for BW, from 0.954 to 0.976 for SL, and from 0.966 to 0.977 for TL, over the two treatments. There no evidence of GxE interaction. There was no significant difference in terms of variation and rank order of performance of strains over the treatments, providing further evidence of no or weak GxE. Mean condition factor for the HE diet ranged from 2.052 to 2.179, and for the LE diet from 1.824 to 2.186, with no significant differences between strains and treatments. Percentage edible yield did not differ between the strains and treatments however; the HE diet resulted in a greater yield than the LE diet. In conclusion, Strain 4 can be recommended for use in production systems that feed HE diets similar to the diet used in this study. No specific strain can be recommended for use in production systems that feed LE diets similar to the LE diet used in this study. The absence of a significant GxE interaction over the treatments also provide an indication that there is no need for establishing separate breeding programs for Nile tilapia for selective use in high and low input systems in Africa.



## **COMMUNITY BASED LAKE RESOURCES MANAGEMENT: A SUCCESSFUL MODEL IN LAKES OF POKHARA VALLEY, NEPAL**

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Nepal is rich in natural water resources and fishing is a long standing tradition in these waters. This paper mainly focuses on eutrophic lake Phewa (500 ha.) and highly eutrophic lake Rupa (100ha.) which are located in Pokhara valley, western mid hill of Nepal. Unplanned developmental activities, environmental changes, lack of proper management, these lakes are environmentally degraded threatening the aquatic biodiversity and livelihood of traditional dependents.

Community were encouraged and has realized about the importance of lake resources and form a legal community base organization (CBO) in Lake Phewa (300 members) and Lake Rupa 670 members, which act as a community base participatory co-management approach. Active participation of communities in cleaning the aquatic weeds, protection of spawning ground and habitat of local fishes, awareness campaign directed to prevent water pollution, stocking of fish in open water for capture fisheries. Besides these activities CBOs have launched eco-friendly plankton base cage aquaculture program in lake Phewa and producing 150 mt.(2014) fish from 650 (5m+5m+2m) floating net cages for their livelihood. The cooperative of lake Rupa after failure of cage aquaculture due to high infestation of aquatic plant over the lake, cleaning of aquatic weed and stocking of herbivorous fish control the weed and increase open water fish production from 6mt(2000) to 65mt(2008) and 90mt(2014). This income from fish is shared among cooperative member as well as to support in social welfare, education for disabled and conservation of Lake. These lakes are common property and are assessable for all; therefore, stakeholders prioritize their role and responsibilities and clear understanding among themselves. Community based participatory management not only supports livelihood of poor section of community living around the wetland but also contributes in maintaining aquatic ecosystem and conservation of biodiversity in sustainable way.

## ECONOMIC ANALYSES OF LAND BASED FARMING OF SALMON

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Salmon farming has been one of the fastest growing industries in Norway for more than three decades. Nevertheless, in recent years the environmental sustainability of the industry has been questioned. This is mainly due to two reasons. First, the incidence of sea lice has increased tremendously, causing diseases and very high treatment costs. Second, escaped salmon mix with wild salmon with undesirable genetic effects. As a consequence of these problems, the government has decided that for the time being no further licenses for sea based salmon farming will be issued which will prevent the industry from further expansion.

This situation has led to increased interest for development of new technology and new ways of growth for a promising industry. This paper presents economic analyses of two modes of production that both represent potential ways for the industry to grow without new licences or an increase in maximum allowable biomass (MAB). They are 1) full land based production cycle and 2) post smolt production – extension of the land based production phase of salmon from normal release of 100 g smolts to a weight at release of up to one kg which will greatly reduce the length of the sea phase. In land based farms the fish are totally protected from the environment and there will be no releases from the farms. The technology is safe with respect to escapes, which means that increased use of the technology will protect wild salmon from genetic interference from farmed salmon. Land based farms are currently being developed in several countries including Norway, Denmark, Poland, Canada and the USA.

The project presents economic analyses of these two modes of production with comparisons with traditional land based farming. If the new systems are competitive, there may also be development of salmon farms closer to main consumer markets than what is the situation today.

## FIELD TESTING A NEW DIAGNOSTIC ASSAY FOR *Amyloodinium ocellatum*

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*Amyloodinium ocellatum* is a parasitic dinoflagellate that causes “velvet disease” in warmwater marine and estuarine fishes. The life cycle is direct and consists of three stages – a trophont that is parasitic primarily on the gills, a free-living reproductive tomont, and an infective dinospore. Because the life cycle is direct, with each tomont producing up to 256 infective dinospores, heavy infections build quickly in aquaculture and are difficult to detect prior to the onset of morbidity using standard microscopy. As a result, the disease is one of the primary impediments to warmwater marine aquaculture. Although a PCR for detecting the parasite has been available since 2007, an easier, more rapid Loop Mediated Isothermal Amplification (LAMP) assay for detection of the parasite was recently developed. Laboratory tests demonstrated that the new assay was highly specific to *Amyloodinium ocellatum* (i.e., did not cross-react with four closely related species) and exceptionally more sensitive than the standard PCR (i.e., could detect as little as 10 fg DNA vs. 1pg DNA for PCR) for detection of the parasite.

Although initial tests indicated that the assay was not affected by samples of water or fish gill tissue collected from outside the laboratory, the efficacy of the assay in improving production outcomes in aquaculture facilities is indeterminate. Therefore, we are cooperating with producers of clownfish(es) (*Amphiprion* spp.) in Florida to field test the new LAMP assay. Paired production systems at a minimum of two farms will be sampled weekly for both standard microscopy and LAMP from prior to stocking through the production cycle to assess the degree to which LAMP detects the parasite earlier than standard microscopy. To establish sensitivity, LAMP and microscopy samples from systems that test positive and negative will be subsampled and tested for the degree to which the results agree. The false positive/negative rate will be assessed using known negative water from the farms spiked with a range of known numbers of dinospores of *A. ocellatum*, repeatedly subsampled, and analysed by LAMP. Differences in production outcomes in systems diagnosed via the LAMP assay and standard microscopy will be compared and a cost-benefit ratio will be developed.

We will statistically evaluate the diagnostic power of the LAMP assay by comparing it to the gold standard of microscopic identification using a 2X2 contingency table analysis. Counts of known (spiked) true positives and negatives and false positives and negatives from each test will be used to calculate sensitivity and specificity and their 95% confidence intervals. McNemar’s test for paired data (i.e., both tests performed on same specimen) will be used to test whether or not the sensitivity and specificity of the two tests are significantly different. Further, we will estimate the reproducibility of the test by evaluating replicates from each prepared sample of known (spiked) positive and known negative prepared samples.

## **A CASE STUDY OF INVESTOR IGNORANCE IN THE RISE AND FALL (AND RISE?) OF THE SOUTH AFRICAN MARINE FISH FARMING INDUSTRY**

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Aqua Management Systems operates a pilot scale, land-based marine fish farm in the East London Industrial Development Zone in South Africa. After applying world-leading technology and operating for over seven years at total investment cost of US\$3.2 million, the operation has had significant investor challenges in the form of board room shootings, international Ponzi scheme implications and drawn-out liquidations – all the while working on overcoming the technical challenges involved in such a pioneering project. Despite everything, the facility has made significant technical advances over the period, proving (and bettering) the majority of the animal growth performance indicators it originally set out to prove. It is now ready to commercialize its operation, but confidence in the aquaculture investment community has been shaken by productivity challenges experienced elsewhere in the South African marine fish farming community.

Aquaculture operations should not to be judged by outward appearances. The financial performance of any aquaculture business is directly dependent on animal growth performance (i.e. growth rate, stocking density, FCR) and market price. So, what should the investor be looking out for to identify an aquaculture business with real potential?

This case study account delves into the complicity between developer optimism and investor ignorance in the underperformance of the South African marine fish farming industry. It will provide investors with some simple tools needed to navigate the quagmire of aquaculture failures in the region, and hopefully instill some confidence in reorienting the industry towards achieving its development potential.

## **A CASE STUDY OF INVESTOR IGNORANCE IN THE RISE AND FALL (AND RISE?) OF THE SOUTH AFRICAN MARINE FISH FARMING INDUSTRY**

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Aqua Management Systems (AMS) is an aquaculture system design, equipment supply and farm operator. Company staff have a successful track record spanning more than twenty years of operation in southern Africa, managing commercial scale marine aquaculture and large public marine aquarium facilities. For support, we maintain strong relationships with internationally reputable aquaculture system design and equipment supply companies.

The financial performance of any aquaculture business is directly dependent on animal growth performance (i.e. growth rate, stocking density, FCR) and market price. We provide on-farm solutions to ensure optimal animal health, conditioning and biomass development – all aimed at achieving positive financial returns.

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## COMMERCIAL IMTA WORKS: LAND-BASED INTEGRATED AQUACULTURE OF SEaweEDS WITH MARINE ANIMALS IN SOUTH AFRICA

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A considerable literature has been produced on the potential benefits, particularly with regard to sustainability, of integrated multi-trophic aquaculture of marine organisms, but there are few examples of systems which have functioned fully commercially over a long time period. IMTA has been fully commercial in South African land-based systems, growing the green seaweed *Ulva* in abalone effluent for feed production, for 15 years. Reasons for this success will be analysed and discussed.

These systems have been produced semi-independently on a number of farms, and have been shown to pay for themselves rapidly. This success has been attained despite the obvious drawback of requiring the expertise to grow two crops, the need for space for seaweed growth, and fears about biosecurity with the movement of materials from different parts of the system. These systems have the potential to reduce the usage of fish-meal based compound feed, as well as the harvesting of kelp from natural forests. In studies with both abalone and urchins, *Ulva* included in the diet has shown numerous benefits in enhanced feed attraction, increased feed consumption, and in improving colour and taste of products, and there is evidence for improved immune system response and beneficial effects on the gut flora of the animals.

The biggest threat to future IMTA in these successful land-based systems is undoubtedly biosecurity fears. Partial re-circulation of effluent using *Ulva* for bioremediation was successful for a number of years on one farm, who have now ceased water re-circulation because of these concerns, although a different farm has instituted partial re-circulation using *Ulva* as a biofilter. The evidence suggests that micro-organisms associated with seaweeds are specific to them, but despite long-term IMTA without problems, microbial studies are necessary to fully allay biosecurity fears.

## NUTRITIONAL CONTROL OF GROWTH, GUT MICROBIOME, AND INTESTINAL NUTRIENT TRANSPORTERS IN NILE TILAPIA

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Global production of farmed Nile tilapia (*Oreochromis niloticus*) has increased exponentially over the past 30 years. Feed comprises 50-70% of production costs for tilapia. Here we assessed if reduced feeding might improve feed efficiency of tilapia grown in ponds in Bangladesh by utilizing pulsed feeding strategies along with weekly pond fertilization [fed daily (Tx1), fed alternate days (Tx2), fed every third day (Tx3), not fed (Tx4)] and without fertilization [fed daily (Tx5)]. Tx1, Tx2, and Tx5 had the greatest growth and survival, while Tx2 had the best feed efficiency and overall benefit:cost ratio of all groups. Metagenomics studies were designed to establish gut microbial diversity changes due to these pulsed feeding strategies. We obtained about 20 million total reads aligning to 225 different prokaryotic (16S operational taxonomic units) and 288 eukaryotic genus/species (18S taxinomial units). Metagenomics analyses indicated that Tx1 and Tx2 had the greatest diversity of bacteria and eukaryotes in the tilapia fecal material. The predominant bacteria found were *Cetobacterium somerae* (common gut colonizers of Nile tilapia) which are known to produce Vitamin B12. Six unique species were found in Tx2 including members of family Nocardioideae (shown antimicrobial and antitumor effects), *Bacteroides* sp. (shown to directly modulate the gut function of their hosts), and *Sphingomonas* sp. (produce antioxidant compounds). The predominant eukaryotes in the tilapia fecal material were the diatoms, rotifers, green algae and flowering plants (angiosperms). Gene expression of solute transporters found in the proximal intestine in the Tx2 regime tended to be higher than feeding alone, but lower than the other feeding + fertilization and fertilization alone regimes. This intermediate expression of transporters with alternate day feeding may reflect a condition for most efficient uptake of nutrients from the GI tract of tilapia. Overall, the results indicate that feeding tilapia on alternate days in fertilized ponds can provide significant cost savings to tilapia farmers with little impact on fish growth and that this regime increases the diversity of microbiota available to the fish and regulates nutrient uptake, which may contribute to the improved efficiency of tilapia growout. This is the first description of the tilapia microbiome derived from next generation sequencing techniques and should serve as a good reference for future studies aimed at evaluating changes in gut fauna and flora linked to tilapia health and performance under different environmental conditions. To build on these studies, experiments have been initiated to determine if larval nutritional conditioning might modify the gut transcriptome and microbial community in favor of improved efficiency. We have conditioned newly hatched fry on an initial 25% crude protein diet versus a usual 48% crude protein larval diet for different interval to determine whether reducing the amount of crude protein in early fry life will lead to subsequent improvements in protein processing, uptake, and utilization in tilapia during growout. We will discuss how differences in gut gene expression and microbial content may contribute to nutritional imprinting in fish, should the phenomenon be observed.

Funded by the AquaFish Innovation Lab of the United States Agency for International Development (USAID) Cooperative Agreement No. EPP-A-00-06-00012-00 and by U.S. and Host Country partners.



## **POLICY AND REGULATION PROCESSES TO SUPPORT AQUACULTURE INDUSTRY DEVELOPMENT IN NSW, AUSTRALIA**

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Attracting new investment to the Australian aquaculture industry is challenging. Reducing business investment risk has been a key goal of Australian states and territories to promote viable and sustainable aquaculture development. At a national level this is being driven through the Australian Aquaculture Committee, state/territory aquaculture managers and the National Aquaculture Council with an aim to streamline approval processes, promote industry best practice, ensure adequate environmental monitoring and provide greater security for businesses.

NSW has embraced the above aim and has developed or is developing a range of industry sector strategies to achieve greater aquaculture investment in the State. This talk will focus on the policy and regulation processes that NSW has undertaken to stimulate aquaculture investment.

## LIMING OF SHRIMP AQUACULTURE PONDS

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Agricultural limestone, burnt lime, and hydrated lime are commonly applied to shrimp ponds. The primary benefit of agricultural limestone is to neutralize acidity of pond bottom soils and increase the alkalinity of pond water. Burnt and hydrated lime also can be used for this purpose if applied to bottom soils between crops. In addition, burnt and hydrated lime are used to increase bottom soil pH and destroy unwanted organisms including disease organisms and their vectors. Burnt and hydrated lime also are sometimes applied to shrimp ponds in small (10-50 kg/ha), frequent doses. This is done in an attempt to remove carbon dioxide and phosphorus from the water, limit phytoplankton growth, and stabilize pH near 8.0.

Many shrimp ponds have waters that are at or near calcium carbonate saturation, and liming materials will not dissolve. Pond bottom disinfection requires at least 2,000-3,000 kg/ha of lime applied while soil is still saturated with water. Most farmers use a much lower lime treatment rate and make the application after pond bottoms have completely dried. There is no evidence from research that small, frequent applications of liming material are effective in improving pond water quality.

The effort and expense of applying liming materials to shrimp ponds often is for naught. Shrimp farmers should pay more attention to whether their ponds require liming, the kind of liming material and rate to use, the quality of liming material purchased, and to having a realistic expectation for the effects of liming. The use of alkalinity and pH measurements, the calcium carbonate saturation index, lime requirement test, and neutralizing value and fineness of liming materials in improving the practice of liming in aquaculture will be discussed.

## ESTIMATING AERATION RATE IN AQUACULTURE

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Mechanical aeration is a standard practice in many types of feed-based aquaculture. However, the determination of the amounts of aeration that should be used in aquaculture operations is still determined mainly by managerial decision based on previous experience. This method of determining aeration rate can result in insufficient aeration leading to low dissolved oxygen concentration or oxygen depletion causing stress or mortality in the culture animals. Of course, excessive aeration leads to greater production costs.

A technology-based method for determining the appropriate amount of aeration is applied in wastewater treatment. This method requires data on efficiency of aerators (from standard oxygen-transfer tests), information on hourly oxygen demand, the target dissolved oxygen concentration, and the quality of water in the aeration basin. A method for estimating the oxygen demand imposed by aquaculture feed was developed, and a procedure for estimating the hourly oxygen demand in aquaculture systems has been devised. The standard aeration efficiency of aquaculture aerators usually is available from manufacturers. This information and aeration equations from wastewater engineering applications can be used to calculate aeration rate for aquaculture systems.

## LIMING AQUACULTURE PONDS

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Although liming is a common practice, the principles of liming are often poorly understood. The main purpose of liming is to increase the pH of extremely acidic ponds and to increase the alkalinity in ponds with low alkalinity water. There are three basic types of liming materials, agricultural limestone, burnt lime, and hydrated lime. These substances are not highly soluble and will not dissolve in water that is already saturated with calcium carbonate. A table of pH values at which water of different calcium concentrations is saturated with calcium carbonate has been prepared and will be discussed.

The most widely used liming material in aquaculture is agricultural limestone, and its quality is based on its neutralizing value and the fineness of its particles. Pure calcium carbonate is assigned a neutralizing value of 100% for comparison and particles that pass a 60-mesh screen are most effective. A lime requirement method for aquaculture ponds also will be presented. It is based on determining the amount of calcium carbonate needed for neutralization of acidity in bottom soil of a pond. It is important to understand that liming is not a fertilization procedure. However, liming to obtain an adequate pH and alkalinity is essential for effective pond fertilization. Burnt or hydrated lime can be used to disinfect pond bottoms, but application rates of 3,000-5,000 kg/ha are needed.

## INCLUSION OF CARBOHYDRATE IN DIETS FOR JUVENILES OF PIRARUCU, *Arapaima gigas*

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Pirarucu (*Arapaima gigas*) is one of the largest scaled fish in the world and has as its natural habitat the floodplain lakes and flooded forests of the Amazon Basin. It presents a carnivorous food habit and its biological and zootechnical characteristics allow to reach more than 10 kg in a year of cultivation. Carbohydrates are the lowest energy source in a diet, but there is a limit to the use of energy from these nutrients, and there is a need to establish the best protein:carbohydrate ratio for cultured fish. The present study was conducted to avaliar o growth performance of pirarucu fed with diets containing increasing levels of carbohydrate inclusion. An 8-week feeding trial was conducted in a water recirculating system with 200 pirarucu juveniles (mean individual weight of  $182 \pm 3.6$  g), stocked in 20 tanques circulares. Water was recirculated through biological and mechanical filters. Water temperature was maintained at 26-28°C. Ammonia, nitrite, dissolved oxygen, temperature, and pH were measured twice a week. It were evaluated five levels of inclusion of non-structural carbohydrates (22, 24, 26, 28 and 30%) in isoennergetic (3600 Kcal/Kg digestible energy) and isoprotein (36% digestible protein) diets containing corn meal, wheat bran, soybean meal, corn gluten meal and corn starch, as sources of vegetable origin, as well as fish meal, chicken viscera meal and hydrolyzed feather meal. The concentration of non-structural carbohydrates significantly affects the performance of pirarucu juveniles (table 1). The results indicate that as the inclusion of non-structural carbohydrates in the diet was increased, a linear effect on feed intake and weight gain was observed. The feed conversion had quadratic effect, being the best result obtained with the inclusion of 25.34%.

Table 1. Performance of pirarucu juveniles fed different levels of inclusion of carbohydrates

| Variable                   | Levels of inclusion of carbohydrate (%) |        |        |        |        | CV    | P value |
|----------------------------|---|--------|--------|--------|--------|-------|---------|
|                            | 22                                      | 24     | 26     | 28     | 30     |       |         |
| Final length (cm)          | 39.28                                   | 40.03  | 39.58  | 39.36  | 38.14  | 2.83  | NS      |
| Weight gain (g)*           | 315.73                                  | 347.00 | 310.94 | 293.98 | 287.80 | 6.38  | 0.0034  |
| Feed intake (g)*           | 272.82                                  | 278.34 | 260.82 | 246.78 | 256.28 | 6.23  | 0.0248  |
| feed conversion**          | 0.86                                    | 0.80   | 0.84   | 0.84   | 0.89   | 3.37  | 0.0028  |
| Specific growth rate       | 0.16                                    | 0.17   | 0.16   | 0.16   | 0.14   | 11.84 | NS      |
| Somatic viscera index (%)* | 10.64                                   | 9.85   | 9.95   | 9.56   | 8.85   | 6.95  | 0.0025  |

CV: coefficient of variation

\* Linear effect

\*\* Quadratic effect

NS: Not significant

# DETERMINATION OF BENTHIC EFFECTS FROM EXCESS ORGANIC ENRICHMENT: ALTERNATIVE METHODS AND TEST APPLICATION NEAR ATLANTIC SALMON *Salmo salar* AQUACULTURE NET-PENS

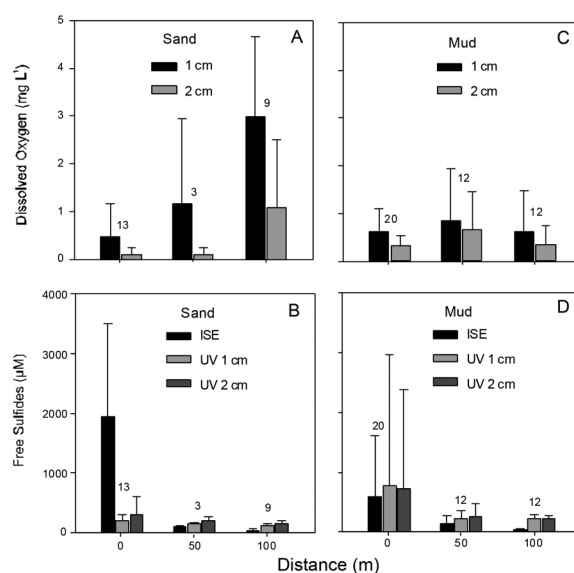
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Increasing organic matter inputs to sediments have a known effect on benthic faunal communities due largely to oxygen depletion and toxic sulfide production. Total dissolved sulfide (free  $S^{2-} = H_2S + HS^- + S^{2-}$ ) concentrations in sediments are widely measured as a practical indicator of benthic organic enrichment effects. However, the accepted ion selective electrode (ISE) protocol for sediment analysis often provides biased results. Alternative protocols for rapid field (UV spectrophotometry) and laboratory (colorimetric microplate) analysis of free  $S^{2-}$  were developed and validated that eliminate interference from non-toxic mineral sulfides and prevent the oxidation and volatilization of free  $S^{2-}$ .

Sediment cores were collected at two Atlantic salmon (*Salmo salar*) farms in eastern Canada (fine mud and coarse sand sediment types). Cores were collected at distances of 0, 50 and 100 m from cage edge, and sediment porewater was extracted from 1 and 2 cm depths below the sediment-water interface. Porewater replicate samples for free  $S^{2-}$  were immediately analysed by the UV spectrophotometric method and fixed for later analysis by the colorimetric microplate method. Dissolved oxygen (DO) was also determined in the porewater. Cores were then subsampled from 0 to 2 cm depth for free  $S^{2-}$  analysis by the ISE protocol.

Results confirm previous conclusions of bias in ISE measurements and more accurately document the spatial extent and magnitude of organic enrichment effects.



**FIGURE 1.** Average ( $\pm 2$  SE) total free  $S^{2-}$  and DO concentrations in surface sediments collected at different distances from net-pens. Sample sizes at each location (number of replicate cores) are shown.

## HORMONAL THERAPIES APPLIED TO THE REPRODUCTION OF *Astyanax altiparanae* (CHARACIFORMES: CHARACIDAE) IN CAPTIVITY, IN WINTER

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In most fish migratory species, vitellogenesis advances in captivity but there is no migration of the germinal vesicle and ovulation, therefore hormonal induction is required to trigger reproduction. *Astyanax altiparanae* is a migratory species that spawn in spring and summer, however, recent studies have demonstrated the ability of this species to reproduce in winter, in captivity, which can optimize production by reducing the off-season period. The objective of this study is to understand the dynamic of vitellogenesis and spawning in the winter, analyzing the pituitary-ovaries axis and applying protocols that improve vitellogenesis and final maturation outside the reproductive season.

**Materials and methods:** Two hundred and forty females were placed in 750L boxes in the *Laboratório de Reprodução in CAUNESP in Jaboticabal-SP* under controlled temperature conditions for 21 days. Twenty animals were used in duplicate in each group: 1- Saline (control) at 20°C; 2- Repeated injections of 4mg/g GnRH at 20°C; 3- Repeated injections of 8mg/g GnRH at 20°C; 4- Saline (control) at 30°C; 5- Repeated injections of 4mg/g GnRH at 30°C; 6- Repeated injections of 8mg/g GnRH at 30°C. Ten animals from each group were sampled every 10 days (initial, medium and final). At the end of the experimental period the remaining females were induced to spawning with hCG under the same temperature conditions. Plasma estradiol, GSI, spawning, fertilization and hatching rate were analyzed.

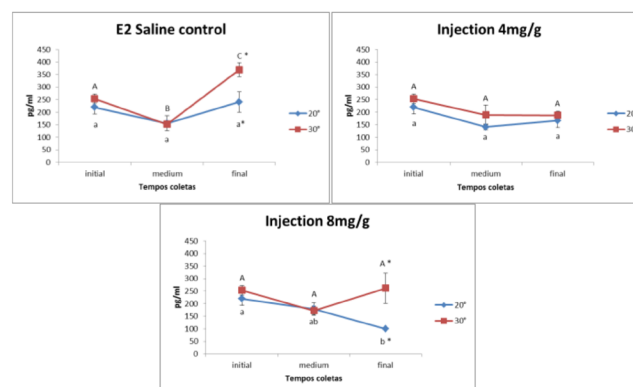
**Results:** Reproduction was successful in all groups induced with hCG, except for group 2. E2 levels increased in females from saline and 8mg/g groups at high temperatures compared to low temperatures.

**Final considerations:** Lower reproductive parameters were achieved in females maintained at 20°C (groups 1, 2 and 3), independently of the treatment. Females treated with the higher GnRH dose at the higher temperature (group 6) resulted in better reproductive parameters, if we estimate the number of larvae, which was probably triggered by higher E2 levels at the final sampling. Thus, even being able to reproduce in the winter, the results showed that the reproductive performance increased with the combination of higher GnRH dose at higher temperature. FSH and vitellogenin levels will be determined to comprehend the physiological modulation of this process.

### Reproductive parameters of *A. altiparanae* females

| Treatment | Number of eggs | Fertilization Rate (%) | Hatching Rate (%) | Estimated larvae number | Accumulated Thermal Units (ATU) |
|-----------|----------------|------------------------|-------------------|-------------------------|---------------------------------|
| 1         | 4.010          | 42                     | 10                | 168.4                   | 330                             |
| 3         | 8.340          | 70                     | 47                | 2743.9                  | 340                             |
| 4         | 7.960          | 92                     | 73                | 5345.9                  | 340                             |
| 5         | 18.360         | 50                     | 50                | 4590.0                  | 140                             |
| 6         | 9.648          | 88                     | 83                | 7046.9                  | 280                             |

Figure: Estradiol levels (pg/ml) in *A. altiparanae* females from saline (control) and GnRH induced (4 and 8 mg/g) groups in different temperatures (20 and 30°C).



**Acknowledgement:** FAPESP (2014/16320-7); CNPq (305779/2015-0; 140428/2016-0).

## THE FINANCING GAP FOR SOUTH AFRICAN AQUACULTURE

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The most significant constraint on the development of the aquaculture industry in South Africa is appropriate investment funding. Aquaculture is a new industry in South Africa and it does not attract significant investment funding from financial institutions. This results in a “funding gap” for developing new opportunities. Financial institutions in particular have little understanding of the risks inherent in the aquaculture sector and how to value opportunities. For the aquaculture sector to reach its full potential, investors will need to have a better understanding of the risks and value creation involved in fish farming. Unless there is a shift in the attitudes of investors and their appetite to fund aquaculture projects, it is probable that the sector will not attract sufficient funds required for the sector to grow. Aquaculturists need to reassess their role in shaping and crafting the investment experience and bridging the funding gap. To this end, this paper will identify significant investment perceptions and make recommendations for aquaculturists to remedy and improve the attitudes of investors towards the sector.



## GENETIC DIVERSITY IN THE SEA URCHIN *Tripneustes gratilla*

Marissa Brink\*, Ruth Dale Kuys, Clint Rhode, and Rouvay Roodt-Wilding

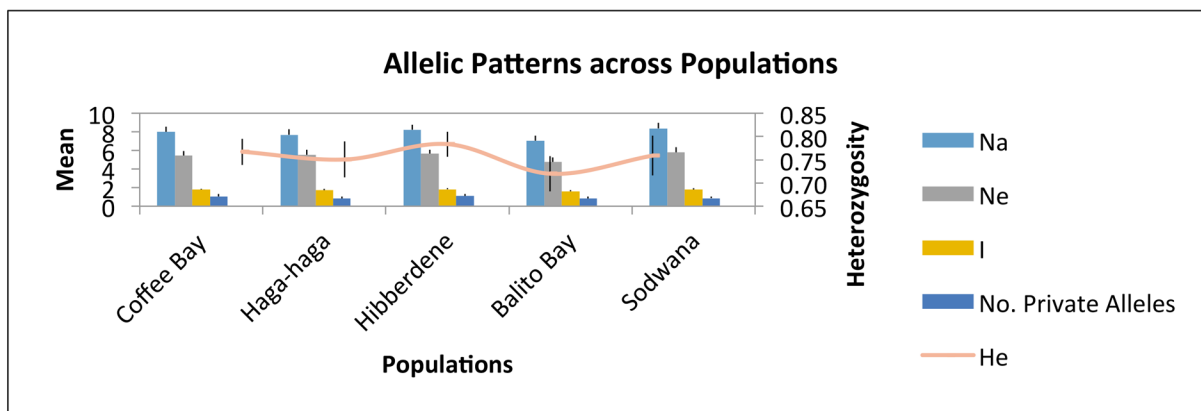
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Sea urchin roe is considered a culinary delicacy in Asia and Europe. The sea urchin, *Tripneustes gratilla*, has been identified as a species with potential for aquaculture production in South Africa. However, this species remains genetically uncharacterized in a South African context. Therefore, the purpose of this study was to provide baseline genetic information that would aid in the establishment of sustainable aquaculture of this species.

Non-destructive sampling methods were developed by using tube feet for DNA extraction, yielding high quality and quantity DNA, comparable to that obtained from roe. Furthermore, natural *T. gratilla* populations were genetically characterized and population connectivity was inferred along the South African coast. A total of 22 species-specific microsatellite markers were used for the genetic characterization of *T. gratilla* samples from Coffee Bay, Haga-Haga, Hibberdene, Balito Bay and Sodwana Bay in South Africa. A low to moderate level of genetic diversity was observed (Figure 1), with a global average number of alleles of 7.86 and an average effective number of alleles of 5.49.

Population differentiation tests suggested that the population representative samples form part of a single large, interbreeding population with  $F_{st}$  values ranging from 0.011 to 0.032 ( $P > 0.05$ ). This can be explained by high levels of gene flow between these geographic locations facilitated by larval dispersal during the planktonic stage.

This is the first study to provide insight into the genetic composition of natural *T. gratilla* populations along the South African coast, and can aid in establishing aquaculture production of this species as well as in monitoring genetic diversity within an aquaculture context in future studies.



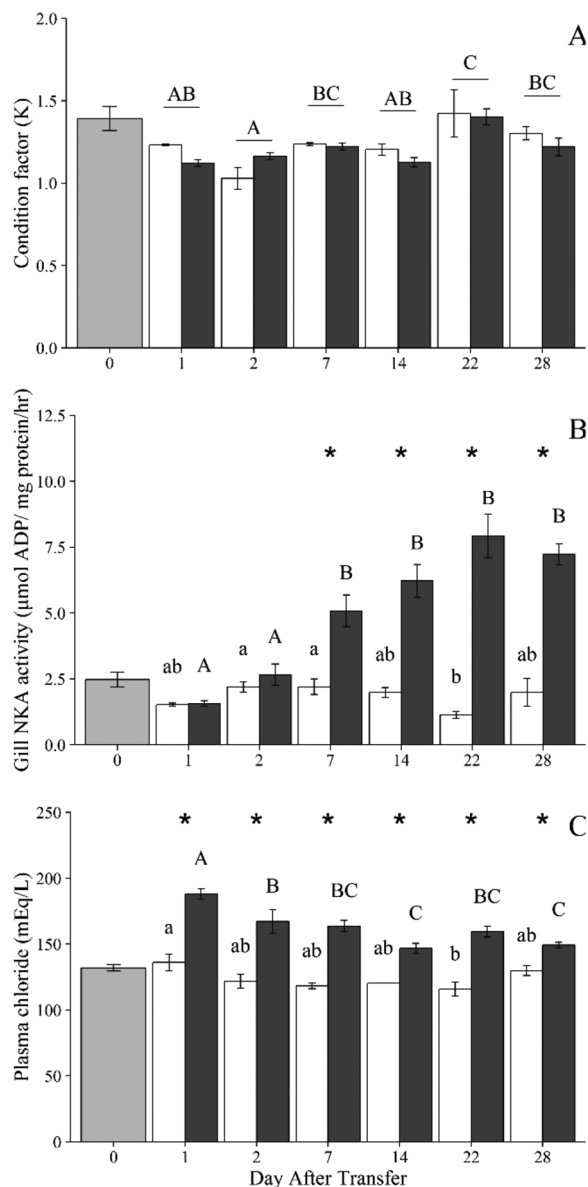
## THE PHYSIOLOGY OF SALTWATER ADAPTATION IN LARGE JUVENILE ATLANTIC SALMON *Salmo salar*

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Smoltification is a critical component of salmonid life history where anadromous species such as Atlantic salmon (*Salmo salar*) undergo physiological, morphological and behavioural changes to facilitate the transition from freshwater to saltwater life. Amongst other changes, Atlantic salmon develop increased saltwater tolerance during smoltification. A period of peak tolerance is maintained during what is known as the 'physiological smolt window'. In juvenile Atlantic salmon (~50g), saltwater tolerance is low outside the physiological smolt window, and a transfer from freshwater to saltwater outside of the window often results in high mortalities or reduced performance. However, in large juvenile Atlantic salmon, salinity tolerance may be independent of smoltification processes.

The present study investigated the effects of transferring freshwater acclimated Atlantic salmon (~678 grams) to saltwater (35ppt) and freshwater (0ppt) on growth and physiological responses over a 28 day period. A high level of saltwater tolerance was observed in fish transferred to saltwater outside of their smolt window, as indicated by 100% survival, similar growth performance to the control and sufficient ionic regulation. Both plasma chloride and osmolality increased rapidly after transfer to saltwater, yet subsequently decreased to new found basal levels 7 and 14 days after transfer respectively, where they were maintained thereafter. The reduction in plasma chloride and osmolality coincided with an increase in gill  $\text{Na}^+/\text{K}^+$ ATPase activity. Plasma cortisol was not different between freshwater and saltwater groups, and plasma thyroxine and lipid content were reduced in saltwater transferred fish for the duration of the trial.



for *Salmo salar* before transfer □ (pre-trial) and after transfer into both freshwater □ and saltwater ■ . Day 0 was not included in statistical analysis

## SPECIAL SESSION ON FINANCING AFRICAN AQUACULTURE

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Africa needs aquaculture. Fish provide 22% of the protein intake in sub-Saharan Africa, but exceeds 50% in the poorest countries (especially where other sources of animal protein are scarce or expensive). In West Africa, the proportion of dietary protein that comes from fish is extremely high: 47% in Senegal, 62%, in Gambia, and 63% in Sierra Leone and Ghana. However, fish supply in Africa is in crisis. Per capita consumption in sub-Saharan Africa is the lowest in all regions and it is the only part of the world where consumption is declining, falling by over 2 kg/pers/year since 1985, driven by stagnant capture fish production and the still-growing population. Just in order to maintain the current supply (6.6 kg/pers/year), African aquaculture has to grow by 267% by 2020.

Models used by the World Bank Group (WBG) show that more poverty alleviation and more equitable economic growth is generated when a larger number of Small and Medium Scale (SME) scale investments satisfy a market, compared to one or a few larger firms. However, in Africa, investment capital for SME businesses is scarce. Without the engagement of formal lending, sectoral growth remains below its potential and only an investment for the relatively wealthy. Another problem associated with the lack of bank engagement is the absence of any leverage for responsible behavior in a sector that has been repeatedly beset by disease and other problems resulting from the failure to adopt best practices.

To assess the approximate scale and financing needs of SME Aquaculture Investments around Africa, a brief survey of currently active farms was carried out over the course of 2016. There seem to be two models, one smaller scale with projected average CAPEX and initial OPEX of around \$1 million, and another medium scale averaging about \$7 million. The overall average is \$4.4 million. These systems cover virtually all types of commercially viable aquaculture technology that has been demonstrated elsewhere.

One thing nearly all of the surveyed farms have in common, is the reliance on personal or family capital with minimal engagement with local banks, which offer only relatively short term loans (<1 year) at high interest rates (~25%). From an initial assessment, most are under-capitalized and few are operating off of professional business plans.

A special session has been organized for the WAS Annual Meeting in Cape Town, RSA to review the status, growth potential and risk profiles of African aquaculture investments, with a focus on the amounts and types of capital needed to help the sector produce revenues, jobs and fish for a growing continent. The session will feature presentations from aquaculture specialists from a range of financial and development institutions interested in African aquaculture, and plenty of time for questions and discussion with a view to helping farmers better understand the constraints for bankers, and helping bankers understand the opportunities in the aquaculture sector.

## UNDERSTANDING SEASONAL PRICE VARIATIONS IN THE AQUACULTURE SECTOR IN UGANDA

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Price fluctuations in Uganda's fish markets have become one of the main risks faced by fish producers. Price movements are for the most part risky, as the direction and force of the motions are largely unknown on a short-term basis, thus complicating production and investment decisions in the aquaculture sector. This paper explores the historical variations in catfish prices and attempts to forecast farm-gate price trends on a monthly basis. The motivation for examining seasonal price patterns and the eventual price forecasts is to allow aquaculture producers to make better-informed decisions and to manage price risk. The forecasts of catfish farm-gate prices are based on historical data from January 2006 to August 2013. Two types of information are extracted from the price data: 1) the monthly price variations relative to the annual average price or the monthly seasonal price indexes, and 2) the price variability within a month during the years included in the analysis.

Seasonal index was computed to measure how much the average for a particular month tends to be above or below the expected value. Figure 1 plot the average annual price and monthly price index, with the variability range indicated by points above and below the index values. For example, for January, the monthly price index of 102.25 means the average January price is 102.25% of the annual average price. The variability factor of 1.95 means that, statistically, the monthly index can vary approximately 2% points higher or lower than the monthly index. Thus, the January price in a particular year may be as high as 104.2% ( $102.25 + 1.95$ ) or as low as 100.3% ( $102.25 - 1.95$ ) of the annual average.

When dealing with aquaculture products, which have a short shelf life, successful forecasting can be an invaluable tool. In this paper, monthly farm-gate prices for catfish are forecasted based on historical data. Of the models developed and tested, a seasonal auto regressive integrated moving average (SARIMA) model outperformed other models in terms of forecasting accuracy on both in-sample and out-of-sample datasets. The results (Figure 2) show that the model can be used to forecast with a mean absolute percentage error (MAPE) of 14% which is considered acceptable for products with stochastic demand.

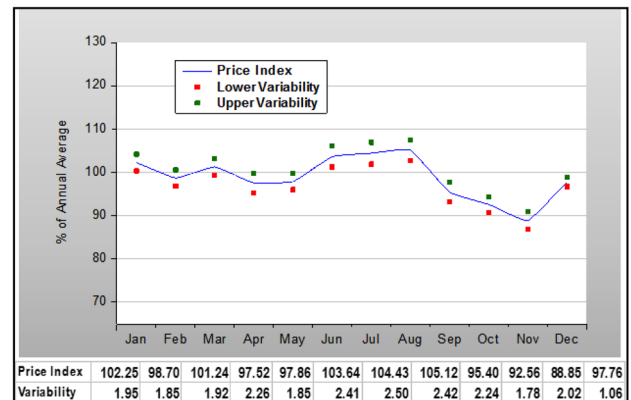


FIGURE. 1. Seasonal Catfish Price Variation (2006-2013)

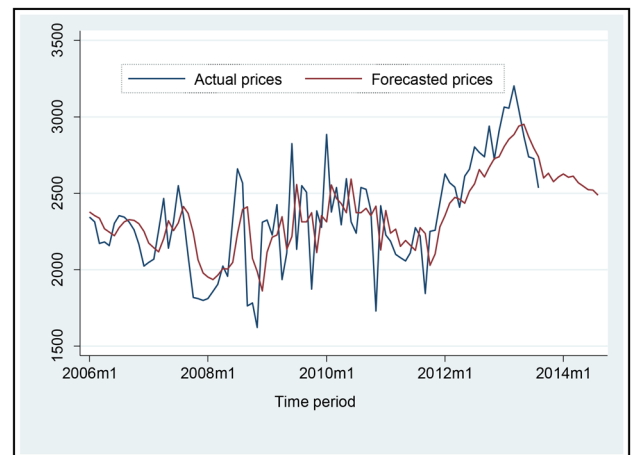


FIGURE. 2. Actual and Forecasted Monthly Catfish Farm-gate Prices 2006:01-2014:08)

## STRENGTHENING POND AQUACULTURE AMONG FISH FARMERS OF WESTERN UGANDA

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Aquaculture in Uganda has the potential to supply the local and regional markets but its ability to meet the production target will depend on increased levels of investment and use of appropriate production technologies. Understanding and meeting the needs of subsistence, small scale, and emerging commercial fish farmers is vital if Uganda is to meet its annual target production levels of 300,000 tonnes of fish. This paper presents findings and recommendations of a study that aimed at understanding the performance of pond aquaculture among fish farmers in Western Uganda. The study was conducted through a semi-structured questionnaire survey and covered 104 fish farmers. Key Informant Interviews were conducted with district production departments' officials. An estimated 20.6% of ponds were abandoned majorly due to failure to generate profits especially after the first production cycle. There was lack of local sources of fish seed so fish farmers obtained their supplies from Kampala and lakes; whereas others stocked once and depended on their natural multiplication while in ponds. There was limited knowledge of on-farm feed mixing among farmers so they depended on feed sourced from feed suppliers which was sometimes of poor quality resulting into low growth of fish and poor harvests. Most small scale farmers who did not receive aid from government programmes and some who ran short of manufactured feed used local materials such as yam leaves and food remains. On average, the fish farmers who majorly used manufactured feeds made losses on tilapia only ponds of Shs -2,683,654 and catfish only ponds of Shs -1,507,236 but made gross profit on mixed tilapia and catfish ponds of Shs 6,604,203 per production cycle. The study recommends that fish farmers should be trained in pond management practices and on-farm making of fish feeds to ensure quality assurance and minimise costs.

### Introduction

Aquaculture is globally considered to be among the fastest growing sectors and, therefore, an important contributor to food and income security. Globally, Production of aquatic animals from aquaculture in 2014 amounted to 73.8 million tonnes compared to capture fisheries which were 90.3 million tonnes (FAO 2016). Africa produces just 2 percent of global aquaculture and employment employs one percent of global fish farmers (Brown, 2012).

In Uganda, Aquaculture was started in the mid 1940s with the aim of improving household food security, nutritional status and people's incomes (MAAIF 2004). However, as a result of increasing pressure on fish stocks resulting from increased population and use of unsustainable fishing gears and methods, Uganda is striving for a competitive, profitable and sustainable fisheries sector as envisaged in the National Fisheries Policy (NFP), the National Development Plan (NDP) 2010/11 – 2014/15 and the Agricultural Sector Development Strategic Investment Programme (DSIP) 2010/11-2014/15 (MAAIF 2004, 2010; MFEP 2010). It is important to note that there has been a significant reduction in fish catch, resulting into decline in fish exports from 36,614 to 23,967 tonnes in 2005 and 2010 respectively (DFR 2012). In order to meet the demand from the increasing population and export markets, Uganda's medium term production target has been set at 300,000 tonnes by 2016, to be met largely through aquaculture development (MAAIF 2010). The sector is estimated to have about 25,000 ponds operated by subsistence, small scale progressive and emerging commercial fish farmers, with estimated farmed fish annual production of 100,000 tonnes (DFR 2012) with Tilapia, catfish and mirror carp being the main cultured species.

Aquaculture has the potential to supply the local and regional markets but its ability to meet the production target will depend on increased levels of investment and use of improved production technologies. These investments will be attracted to the sub-sector if cost-effective and productive technologies that yield high returns at the end of each production cycle are available. Estimates of economic returns are considered essential for intending investors and financial institution like banks to ascertain the risk involved in investing in aquaculture enterprises vis-a-vis other enterprises (Hyuha *et al* 2011).

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This paper presents the findings of study was aimed at assessing the performance of pond aquaculture in order to make recommendations that could enhance which would consequently result into enhanced adoption of aquaculture in Western Uganda.

### **6.1 Key features of fish farmers**

The fish ponds in Western Uganda were managed mostly by the owners (78.4%), although others were managed by farm managers (16.7%) and group chair persons (4.9%). These were mostly males (91.3%) with just a few females (8.7%). Those who belonged to farmer groups were 53.4% while 46.6% operated in isolation. According to Walakira and Atukunda (2011), formation and operation of certain fish farmers' groups in Uganda had directly increased economic and social benefits, especially in rural communities. This is partly because active groups can help farmers in easy accessibility to information, quality feeds, quality seed among others thus resulting into better benefits to fish farmers.

Educational achievement of the majority was incomplete primary (31.7%), followed by tertiary education (24.8%). Other levels were no education (2.0%), complete primary (17.8%), incomplete secondary (12.9%) and complete secondary (10.9%). There is need to improve educational levels among fish farmers to enable them comprehend technical aspects of fish farming and adopt improved technologies, guidelines and best practices. This is more so because most of the information on aquaculture is in English as opposed to local languages. However, given the farmers' current education levels, it is important that information targeting farmers is translated to languages that they can easily understand since at least 98% of respondents of this survey would be able to read local languages.

### **STATUS OF FISH FARMING:**

Western Uganda is a region of great potential for fish farming, given the available water supply. To tap into this potential, all the districts visited had provisions in their District Development Plans (DDP). The major issues focused on in the plans were the quality of feeds and seed as well as general training in fish farming. However, implementation of the plans fell short due to insufficient funds and in some districts inadequate staff.

The levels of fish farming activities varied from district to district with Kasese reporting the highest number of 400 fish ponds while Rubirizi had less than five. A number of the ponds are functional (%) and stocked with mainly Tilapia and Catfish but many are poorly managed due to lack of knowledge and skills. Fisheries Officer often offer advisory services to fish farmers, especially in areas of pond construction/siting and pond size as well as fish feeding and stocking ratios.

Fish farmers source seed from seed suppliers who bring seed from Kampala or from a local hatchery in Bushenyi, called Kabeihura Farmers Ltd. In Mitooma District, farmers continually harvested from the seed which they stocked way back (when). Fish farmers in the district are scattered and there is no specific support given to them because most of the resources are controlled at the sub-county and the little at the District are allocated to only active fish farmers. Most fish farmers use local feeds which include yam leaves, food remains and sometimes maize bran. The use of this type of feed is due to lack of money to buy feed which is considered expensive. Some seed and feeds are also supplied through the NAADS programme and Operation Wealth Creation (OWC).

In Bushenyi, out of the 72 fish farmers in the District, 40 were supported by NAADS with fish seed and feeds. However the feeds were only supplied for the first four months so some farmers resorted to feeding fish with yam leaves, which negatively affected the growth rates of fish. It would be helpful if farmers were trained on making their fish feed on-farm in order to minimize the costs.

In Kabarole, the District has over 300 fish ponds although most of them are new and others have been abandoned. NAADS supported one youth group (10 people) in pond construction, fish feed and seed unfortunately the youths abandoned the enterprise after benefiting from the project. The district is targeting 300 cages by 2021 and so far they have 30 cages on four crater lakes. However, most small scale fish farmers lack knowledge and skills in fish farming and as such they practice aquaculture for subsistence purposes. Essential fish farming training is offered to farmers on pond construction, feeding as well as the likely proceeds from fish farming but there is no clear information on pond production levels due to inadequate resources for field visits to farmers.

*(Continued on next page)*



In Hoima, 10 fish farmers had been supported through OWC with fish feed (7 tonnes) and fish seed of Nile Tilapia (24,000) and Cat fish (28,000) and basic training in fish farming. In the next five years, there are plans to support at least 5 fish farmers every year with fish feed, seed, pond construction and training.

In Masindi, the District supports some fish farmers with fish seed and feed with the limited budget allocated to the Fisheries Department. There is no clear record of existing ponds because the rate of pond abandonment after the first harvest is high. In addition, the staff are constrained by resource availability to carry out adequate monitoring to ascertain the number of ponds and farmers still in production. Some farmers also abandoned their ponds due to theft of fish, high cost of production as well as lack of knowledge and skills in fish farming.

In Kiryandongo, fish farmers feed their fish on yam leaves and food remains which translates into low growth rates. The district lacks feed suppliers so large scale farmers source feed from Kajjansi (what?) and Ugachick and; besides, there are many farmers who would like to be trained in making fish feed on-farm.

### **Type of fish farmed**

Most of the ponds targetted tilapia (60%) but a few were for catfish or mixed tilapia and catfish species (Figure 2). This concurs with ----which reports that Ugandan farmers usually farm tilapia (. The reasons for that could be due to easy accessibility and the fact that interventions usually focus so much on tilapia than Catfish.

### **Pond ownership, construction and management**

Average number of ponds owned were 2. An aveage of the 2 ponds had been harvested by respondents during the last production cycle in 2014/15 after which some ponds were abonded especially after failing to generate profits. In addition, having received aid from especially OWC, some expected more of such aid so they were still waiting for another round of help since they claimed not to have capital to invest in the business.

The average size of the sampled fish ponds was 433 sq M, which was below the size recommended for small scale fish farmers of 600 m<sup>2</sup> (Rutaisire *et al*, 2009). FAO (2016) recommends that the minimum commercial fish size should start at 20X25m (500m<sup>2</sup>) which can produce 120kgs of fish. FAO (2016) recommends larger ponds from 20m to 50 m or more for commercial fish production. Rural farmers need to be helped to start small but progressively advance to commercial status. Suffice to say that small scale fish farmers can contribute significantly to food security as they can provide a source of protein to rural people especially to the pond owners and those in the neighbourhood who would otherwise have found it cumbersome to buy fish in far away markets.

Apart from most ponds being of small sizes, recommendations under pond site selection and construction were well adopted. The most commonly adopted was the use of clean and constant water sources (92.3%), followed by gentle inflow and outflow of pond water by gravity (82.7%) and ensuring ponds were free from residues from human activities (81.7%). FAO (2016) recommends that the most suitable location of land for ponds is a land on a slope next to a flowing stream or river where a portion of the water is diverted into the pond without disrupting the other uses of the same water down stream. That is should be are an area of open and flat land with a gentle slope that allows water to flow by gravity is ideal (FAO,2016). Poorly constructed ponds were observed and this can be attributed to a series of factors which according to ---include the use of pond diggers who have limited knowledge and the impression that fish can grow in a very type of pond. The issue of feed scarcity will usually determine pond sizes, Usually fingerlings are bought once but feeding poses the major challenge. Farmers will have to increase e the z=sizes of ponds when they are assured of availability of manageable or cost effective feed.

Farmers used different methods in fertilizing their ponds to stimulate natural food growth for the fish. On average, respondents who used chemical fertilizers were 5.8%, organic 57.7%, and both chemical and organic 10.6%, while those who did not apply any pond fertilisation were 26.0%. In order to stimulate returns, it's vital that fish farmers are equipped with knowledge and skills in the quantity and type of fertilisers as well as when the fertiliser should be applied. Other practices were record keeping (54.9%) and sampling, sorting and grading of the fish stock regularly (53.9%) and application of measures to control predators (35.3%). Sorting and grading especially for catfish according to Rutaisire (2009) are key activities in fish farming in order to deter some the shooters or jumpers from cannibalising on others and affect the benefits that the farmer would accrue from the fish farming business. Control of predators is equally important if one is the fish farmer is to guarantee benefits. Pond bank slanted to avoid collapsing was the least adopted (51.9%).

(Figure 8).

(Continued on next page)



The period before pond repair began was 1.7 years, equivalent of 2 production cycles. This is too long and it is important to carry out some pond maintenance after every production cycle otherwise, this may be attributed to poor management of ponds.

### **Pond stocking**

On average, respondents stocked more fingerlings in catfish only ponds (2,500) than in tilapia only and mixed tilapia and catfish ponds. The costs per fingerling were uniform for the two species although catfish fingerlings were bigger (50 gm) than those stocked in tilapia and mixed ponds (Table 1). It was also observed that there was lack of local sources of fish seed in the region, save for the single Kabeiura Farms Ltd., located in Bushenyi District and fish farmers had either to obtain their supplies from Kampala or from the lakes, thus compromising the quality. According to Rutaisire (2009), sources of feed should be either government or a trained farmer specialising in fish breeding and seed should be free of disease and parasites. Only 61.8% of respondents complied with recommended stocking rates. About 3% of the respondents stocked tilapia once and relied on natural multiplication of fish so they did not do further stocking.

### **Compare what was stocked and what was harvested to see loss levels/mortality levels**

#### **Feeding of the fish**

Although a few farmers obtained feeds from the shops in their district towns, most respondents made use of manufactured feeds (44.7%) which were mainly provided by OWC and NAADS followed by naturally available food (24.3%) such as yam leaves and food remains. Only 10% of the respondents mixed their own feeds which mainly included maize bran as the main ingredient.

Generally the study established that there was limited knowledge of on-farm feed mixing among farmers although farmers said this would be a better option for them rather than relying on open market feeds whose quality was not guaranteed. Small scale fish farmers also preferred that research be carried out on the materials such as yam and cabbage leaves since some fish were feeding on them and were able to grow and eaten. This argument is not new, a prominent farmer in one of the districts in Western Uganda indicated that using local feeds from fish bones, banana peels, green vegetables, maize bran had given him better results compared to using floating feeds. The same farmer had established that Nile Tilapia was able to eat green leaves of cabbages, dodo and other green leaves and fish is harvested after eight months. Tilapia reared for 10 months fetched Shs 5,000 each while catfish reared for six months would generate Shs 10,000 to Shs 20,000 each (Tibyangye 2013). The prices above are for a period of 2011 but are still below the ones given by the respondents of this study that was carried out in 2016. However, it might not be easy for a rural fish farmer to spend more than a dollar on feeds daily amidst other household demands, so exploiting the potential for using such local materials may reduce the cost of production and increase sustainable stocking and not abandoning ponds. For example a farmer in a rural area may find it easy to grow yams from which he will get yams for his household and also get leaves for his fish. According to Rutaisire et al (2009), if a fish farmer decides to make on farm feeds, the feed must provide all food nutrients required for optimum growth of cultured species. The above notwithstanding, a small scale fish farmer may feel more comfortable rearing fish for an extra two months beyond the recommended six months for Catfish and eight months for Tilapia for as long as the farmer is using local materials to feed the fish though with minimum required nutrients.

The quality of manufactured feeds which affected the growth rate of their fish was however wanting. The data also revealed that only (43.5%) of respondents took note of expired feeds, which could have affected the performance of feeds used. This is more so where people received feeds from programmes such as OWC and usually they received in lumpsum so they were supposed to store the feeds thru a longer period of time. The major challenge with OWC feeds was that fish farmers were able to receive feed that would otherwise been expensive on their side in the first production cycle but could not manage to buy again which would result into pond abandonment. Usually it's the fish farmers who would meet the cost of digging ponds through either hiring labourers to dig the pond or pooling labour as a group to dig a group pond. Digging a pond is not a simple activity so the fact that these people dug the pond showed that they were committed to carrying out fish farming but only needed to be equipped with appropriate technologies to ensure continuity.

#### **Pond harvest and fish sales**

Both complete and partial harvests were practiced among fish farmers. Catfish farmers (60.6%) were more involved in complete harvest while tilapia fish farmers (56.7%) were engaged in partial harvest.

Pond harvest and fish sales data are summarised in Table 2 as follows

*(Continued on next page)*

## 6.2 Fish marketing

Various marketing arrangements existed in the region. In the mid-Western region, farmers relied on the export market to DRC through MPondwe border Post market for their harvests. However, at the time of the study, the farmers were challenged by the flooding of the DRC market with immature fish from the lakes resulting from relaxation of control of the fisheries due to the disbandment of BMUs and suspension of activities of Fisheries Officers, special enforcement officers and police on the water bodies through a Presidential directive. The situation was aggravated by the low adoption of Chorkor fish processing technology among the fish farmers, which would prolong the shelf life of the fish for better marketing.

In the north-Western districts, however, the market remained buoyant, absorbing whatever little fish was available, strengthened by the declining catch from Lake Albert due to overfishing resulting from relaxed control arising from the said Presidential directives.

On average, the fish farmers marketed their harvests once every production cycle.

Marketing costs incurred per production cycle were as follows:

- Cost of packaging materials: Shs 7,000
- Transport charges: Shs 13,500
- Municipal charges: Shs 3,000

Characteristics of fish marketing by fish farmers were as outlined in Table 12. Most farmers sold their harvests in fresh form (96.3%) due to the available ready market (84.6%) and lack of equipment for fish processing (11.5%). The major buyers of fish were direct consumers (83.3%), followed by local traders (12.8%). Most fish farmers sold their harvests at pond side (59.5%) although some fish was sold at the nearby markets (35.4%) within an average distance radius of 1 km.

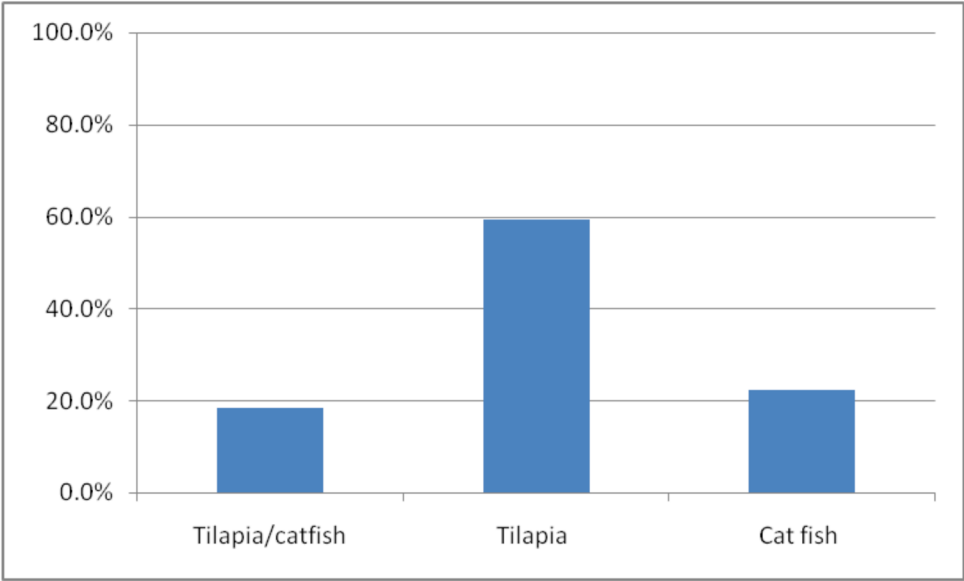
Prices at the away-market locations were on average as below:

- Tilapia (Shs/kg) 6,000
- Tilapia (Shs/ head) 2,500
- Cat fish (Shs/kg) 4,000
- Cat fish (Shs/head) 3,500

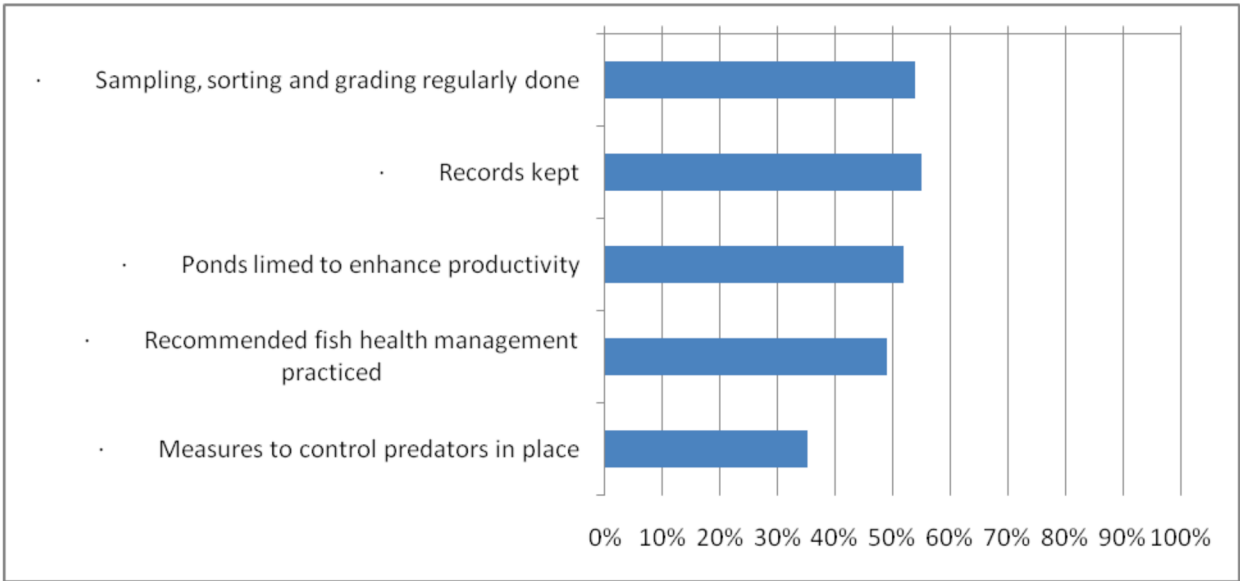
## Conclusion

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**Figure 2: Target species of sampled fish farmers in Western Uganda**



**Figure 8: Adoption rates of technologies, guidelines and best practices in pond management practices**

**Table 1: Average number, weight and cost per fingerling stocked per pond**

|   | Tilapia | Catfish | Tilapia & Catfish |
|---|---------|---------|-------------------|
| Number of fingerings stocked              | 1,511   | 2,500   | 1,553             |
| Average weight of fingerling stocked (gm) | 8.7     | 50.0    | 13.9              |
| Cost per fingerling (Shs)                 | 347     | 350     | 342               |

**Compare what was stocked and what was harvested to see loss levels/mortality levels**

**Table 2: Pond harvests and sales, fish prices and average weight of fish in a production cycle**

| <i>Species of pond</i> | <i>Tilapia</i>             |                       |                   |                          | <i>Catfish</i>             |                       |                   |                          |
|------------------------|----------------------------|-----------------------|-------------------|--------------------------|----------------------------|-----------------------|-------------------|--------------------------|
|                        | Quantity harvested (heads) | Quantity sold (heads) | Prices (Shs/head) | Average fish weight (kg) | Quantity harvested (heads) | Quantity sold (heads) | Prices (Shs/head) | Average fish weight (kg) |
| <i>Tilapia/catfish</i> | 856                        | 987                   | 2,500             | 0.500                    | 1,358                      | 1,250                 | 6,000             | 0.800                    |
| <i>Tilapia only</i>    | 250                        | 200                   | 2,000             | 0.400                    |                            |                       |                   |                          |
| <i>Cat fish only</i>   |                            |                       |                   |                          | 530                        | 520                   | 4,000             | 0.500                    |
| <i>Total</i>           | 375                        | 325                   | 2,000             | 0.450                    | 650                        | 650                   | 4,000             | 0.600                    |

**Costs and revenues**

**Table 12: Fish forms, types of buyers and market destinations for fish farmers**

|  |  |
|--|--|
| Form in which fish farmers sold fish             | <ul style="list-style-type: none"> <li>• Fresh (96.3%)</li> <li>• Smoked (1.2%)</li> <li>• Deep fried (1.2%)</li> <li>• Others (1.2%)</li> </ul>   |
| Why fish was sold in those forms                 | <ul style="list-style-type: none"> <li>• Market availability (84.6%)</li> <li>• Lack of skills/knowledge for fish processing (1.3%)</li> <li>• Lack of equipment for fish processing (11.5%)</li> <li>• Others (2.6%)</li> </ul> |
| Major buyers of fish from the fish farmers       | <ul style="list-style-type: none"> <li>• Consumers (83.3%)</li> <li>• Local traders (12.8%)</li> <li>• Regional traders (2.6%)</li> <li>• Others (1.3%)</li> </ul>   |
| Locations where fish farmers sold their harvests | <ul style="list-style-type: none"> <li>• Pond side (59.5%)</li> <li>• Local market not at the pond (35.4%)</li> <li>• Another District (3.8%)</li> <li>• Regional market (1.3%)</li> </ul>                                       |

Prices at the away-market locations were on average as below:

- Tilapia (Shs/kg) 6,000
- Tilapia (Shs/ head) 2,500
- Cat fish (Shs/kg) 4,000
- Cat fish (Shs/head) 3,500

## EXPECTED GENETIC GAIN FOR SURVIVAL RATE WITH MASS AND FAMILY SELECTION IN THE PRESENCE OF WHITE SPOT SYNDROME VIRUS IN THE PACIFIC WHITE SHRIMP *Penaeus vannamei*

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This study used deterministic simulation to 1) predict genetic gain ( $\Delta G$ ) for survival rate to White Spot Syndrome Virus (WSSV) in *Penaeus vannamei*, and 2) to find the combination of number of families/number of sibs per family necessary to maximize the genetic gain while restricting the inbreeding rate increase per generation to 1%, and by using different population sizes (i.e., number of shrimp used).

Survival in the presence of WSSV was considered as a binary trait (1=alive, 0=dead). The population sizes studied (T) were 1,000, 5,000, 10,000, and 20,000. The average number of sibs per family was derived as the population size divided by the number of families considered. Heritability values considered were 0.05, 0.10, and 0.15. Generations were considered discrete and we used a generation interval of one year. Hence, the genetic gain and the inbreeding rate increase are presented by year. The  $\Delta G$  using mass selection was calculated from  $i\sigma_p h^2$ , where  $i$  is the average selection intensity,  $\sigma_p$  is the phenotypic standard deviation, and  $h^2$  is the heritability of the trait (Falconer y Mackay, 1996). The  $\Delta G$  using family selection was calculated as  $i\sigma_p h r$ , where  $r$  is the accuracy of the predicted breeding value (Cameron, 1997). Calculations were based on the selection index (VanVleck, 1993) and the Saxton approximation (Saxton, 1988) for the selection intensity. Also, with the aim of considering additional adjustments in the genetic variance, selection accuracy, and the inbreeding rate increase per generation as result of selection, the predictions were further calculated using SelAction software (Rutten et al., 2002).

Results show that within each population size there is a number of families that yields the maximum genetic gain and that the number of families associated is different for every T value, with 150 for T = 1000, between 250 and 350 for T = 5,000, between 350 and 500 for T = 10,000 organisms, and of 500 for T = 20,000. The larger genetic gains were observed when using family selection and T > 1,000. Mass selection can be a low cost option to select for increased survival rate with WSSV infestations.

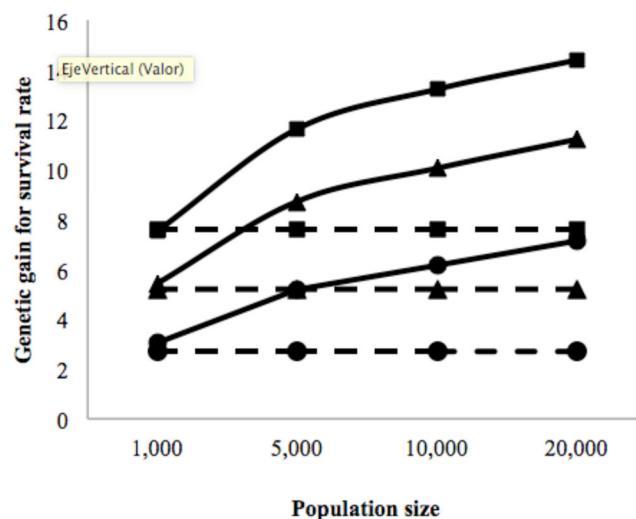


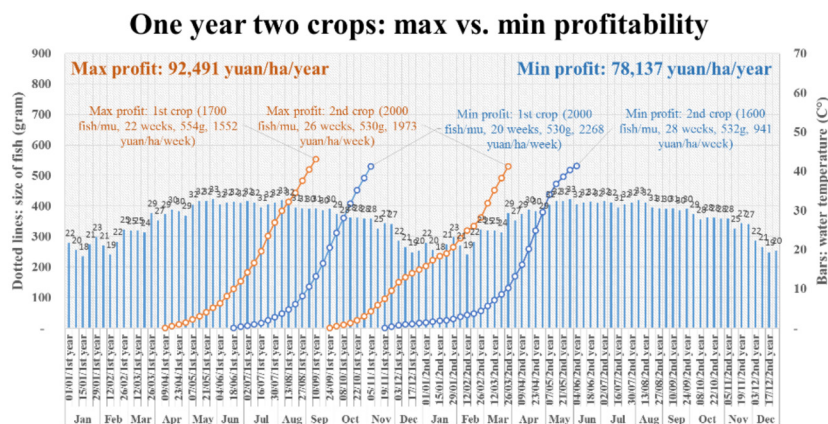
Fig. 1 Maximum genetic gain for survival rate in the presence of WSSV using family selection (solid lines), and mass selection (dotted lines), per population size and heritability value (0.15=squares, 0.10= triangles y 0.05=circles) in the Pacific White Shrimp.

# IMPROVING THE TECHNICAL AND ECONOMIC PERFORMANCE OF TILAPIA AQUACULTURE UNDER CLIMATE VARIATION: PERSPECTIVE FROM BIO-ECONOMIC MODELLING

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Tilapia is the world's most popular aquaculture species farmed mostly in earthen ponds. Experience in China (the largest tilapia farming country) is used to develop and calibrate a bio-economic model on intensive tilapia pond culture. The model is used to simulate the impacts of climate, technical and/or financial factors on farming performance and examine the performance of various farming arrangements under different conditions. The simulation results indicate that 1) an increase in feed price, an increase in mortality, or a decrease in fish price would significantly reduce the profitability and reduce the optimal harvesting size; whereas an increase in the price or cost of seed, labour, rent, electricity or water management would have smaller impacts on profitability; 2) considering the impact of water temperature on fish growth, the profitability of a production cycle starting at the best timing could be twice as high as one starting at the worse timing; 3) farming arrangements that maximize the profit of individual crops may not maximize the overall profitability because of path dependency of farming performance; 4) farming arrangement that maximizes the overall profitability can significantly improve the economic performance (see the figure on the right); 5) given no price discrepancy against small size fish, harvesting at about 300g in two-year-five-crop arrangements could increase the overall profitability by up to 50 percent compared to harvesting above 500g in one-year-two-crop arrangements; 6) a two-tier farming system that separates nursing and outgrowing ponds could allow one-year-three-crop arrangements that enhance profitability by up to nearly 90 percent compared to the one-year-two-crop arrangements and by up to nearly 30 percent compared to the two-year-five-crop arrangements. With more refined information on fish growth under different farming conditions, the model could become a decision-making tool to help farmers design optimal farming arrangements.





## ASSESSMENT AND MONITORING OF SPECIES DIVERSIFICATION IN GLOBAL AQUACULTURE

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Species diversification in aquaculture has been driven and influenced by various factors, including consumer preference, technical innovations such as advances in artificial propagation, economies of scale, risk diversification, policy orientation, among others. Global aquaculture development in general has been associated with species diversification in terms of both increasing number of farmed species and more even distribution of production among species, yet diversifying patterns vary across countries or regions. While species diversification has become a popular notion attractive to policymakers and other experts, there is much room to improve in measuring species diversification in aquaculture and proper use of diversity indicators to facilitate evidence-based decision making. This paper reviews the concept and measurement of farmed species diversity and conducts a global assessment of species diversification in aquaculture based on the FAO global aquaculture production statistics. The assessment covers close to 600 farmed species items registered by FAO in nearly 200 countries or territories for over 60 years since 1950 (see the figure for an example). The assessment quantifies aquaculture species diversity with indicators at various species aggregate levels for different farming environments and/or geographical areas. The assessment synthesizes the status and trends of aquaculture species diversification at the national, regional and global levels. Some stylized facts and salient issues uncovered by the assessment are highlighted and discussed in the paper; whereas all the assessment results are documented in spreadsheet templates for easy access by other experts who are encouraged to uncover stylized facts or salient issues in addition to those addressed in the paper. The spreadsheets are components of the aquaculture production module in the World Aquaculture Performance Indicators (WAPI). Information about WAPI can be found in the Report of the FAO Expert Workshop on Assessment and Monitoring of Aquaculture Sector Performance, Gaeta, Italy, 5–7 November 2012 (FAO Fisheries and Aquaculture Report No. 1063. Rome. 94 pp.).<sup>1</sup>

<http://www.fao.org/documents/card/en/c/ec92bf28-52c4-5f0f-8489-397b41ee223b/>

### Species diversification in global aquaculture (2014)



## AQUACULTURE GROWTH POTENTIAL: PERSPECTIVE FROM SHORT-TERM PROJECTION OF FUTURE FISH DEMAND

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A short-term projection model has been developed to estimate fish demand growth driven by population and income growth and examine the potential of aquaculture in satisfying the demand growth at the national (nearly 200 countries), regional (about 40 country groups) and global levels for 9 species groups at different aggregate levels. The results indicate that population and income growth in the near future (5 years from the mid 2010s to the early 2020s) would drive world fish demand up by over 40 million metric tonnes. Trend aquaculture growth (i.e. aquaculture production in every country follows its recent trend) would be able to cover less than half of the demand growth, leaving an over 20 million tonnes demand-supply gap. Most of the gap is in shellfish and marine finfish species. China accounts for over half of the gap; South-eastern Asia, South Asia and Africa also have relatively large gaps. Trend aquaculture growth in over 150 countries would not be able to cover their fish demand growth driven by population and income growth; in more than 100 countries trend aquaculture growth would cover less than 5 percent of fish demand growth. World aquaculture would need to double its trend growth from 5 to 10 percent in order to fill the demand-supply gap; see the figure for the situation of individual countries. Results for the other 8 more disaggregate species groups are similar qualitatively but vary quantitatively. Results generated by the short-term projection model are useful for policymaking, business planning, development aids and other decision makings by various stakeholders in aquaculture and fisheries. They are complement to and can potentially enhance the understanding of the results of more sophisticated forecasting models such as the OECD-FAO Fish Model and the WB-IFPRI-FAO Fish to 2030 model.



## ASSESSMENT AND MONITORING OF GLOBAL TILAPIA MARKETS

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Seafood is the most traded food commodities. Tilapia is the most popular aquaculture species farmed in over 100 countries. Tilapia is one of the most traded seafood commodities. This paper conducts an in-depth analysis of the international markets of 4 major tilapia commodities (fresh whole, frozen whole, fresh fillet and frozen fillet) as well as their aggregates at the national (country or territory), regional (including geographic regions, trade blocks and ad-hoc groups) and global levels. As tilapia import statistics are not readily available in all tilapia importing countries, we supplement import statistics with export statistics in order to account for global tilapia markets more comprehensively. We examine the status and trend of global tilapia markets in 2013, 2014 and 2015 (the latest years with available data), with focus on assessment of the status in 2015 and monitoring of the trend between 2014 and 2015. We use a suite of indicators to assess and monitor tilapia international markets, including indicators of basic market conditions (e.g. market size and price), those of market structure (e.g. number of suppliers and effective number of suppliers) and those of market potential (e.g. share of frozen whole tilapia in all frozen whole fish and per capita tilapia import). Key results for frozen whole tilapia markets include 1) at least 133 countries (or territories) imported frozen whole tilapia in 2015 (see the figure); the total market size is 345 million US\$ and 172 thousand tonnes; both declined slightly compared to 2014; 2) while many countries in Africa are concerned about the impacts of cheap tilapia imports on domestic tilapia farming industries, it appears that the prices of frozen whole tilapia in most African markets, as well as the average price in African markets, are higher than the world average (2 US\$/kg in 2015); 3) frozen whole tilapia markets in developed regions generally have more suppliers than those in developing regions—while the United States imported frozen whole tilapia from 17 countries, over 50 national markets in developing regions imported the commodity from only one country; 4) despite the largest number of suppliers (17), the effective number of suppliers to the frozen whole tilapia market in the United States is only 2.8, indicating high market share concentration; 5) relatively low share of frozen whole tilapia in total frozen whole fish import as well as low per capita import indicates great market potential for the commodity in many countries. Similar salient issues and stylized facts for all the four tilapia commodities are highlighted and discussed in the paper. All results (including those for aggregate commodity groups) are presented in user-friendly spreadsheets as appendix of the paper. The spreadsheets are components of the fish trade module in the World Aquaculture Performance Indicators (WAPI). Information about WAPI can be found in the Report of the FAO Expert Workshop on Assessment and Monitoring of Aquaculture Sector Performance, Gaeta, Italy, 5–7 November 2012 (FAO Fisheries and Aquaculture Report No. 1063. Rome. 94 pp.).<sup>1</sup>

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**Global markets of frozen whole tilapia in 2015**



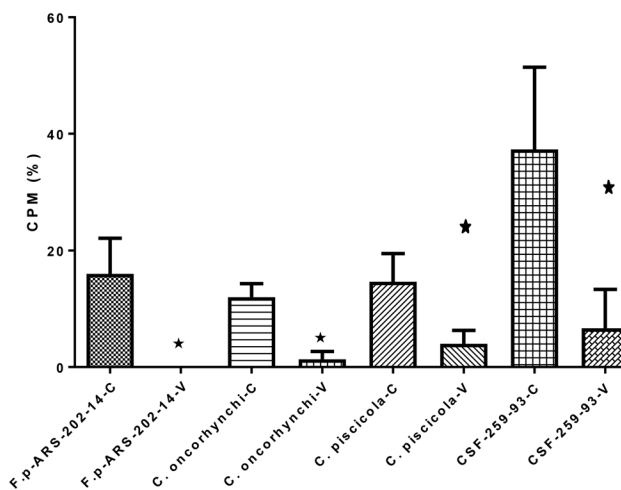
## CROSS-PROTECTION TO EMERGING FLAVOBACTERIAL PATHOGENS FOLLOWING VACCINATION WITH A LIVE ATTENUATED *Flavobacterium psychrophilum* VACCINE (FP-B.17-ILM)

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A series of trials were carried out to determine the level of cross-protection provided by a live attenuated vaccine (FP-B.17-ILM) developed to protect fish from coldwater disease and rainbow trout fry syndrome (CWD/RTFS). In the initial vaccination trial, four pathogenic flavobacterial isolates from clinically diseased fish sampled at a commercial steelhead trout (*Oncorhynchus mykiss*) growout site were tested. These included one presumptive strain of *F. psychrophilum* (ARS202-14), one unknown *Flavobacterium* sp. (2014-9172-1-12) and two species of *Chryseobacterium* (*C. oncorhynchi* and *C. piscicola*). Fish were injection vaccinated with the FP-B.17-ILM vaccine and then immersion challenged with the test isolates or injection challenged with a positive control strain of *F. psychrophilum* (CSF 259-93). Mortality in challenged fish (35g/f mean wt) was generally low but solid cross-protection was observed against *C. oncorhynchi* and *C. piscicola*. Protection to the ARS202-14 strain of *F. psychrophilum* and the *F. sp* (2014-9172-1-12) was inconclusive due to low mortality following challenge. Therefore, a follow up trial was conducted using smaller fish (23g/f mean wt at challenge) immersion challenged with higher doses of select strains. Results from this second trial showed that vaccinated fish had significantly lower cumulative percent mortality (CPM), and relative percent survival (RPS) values ranged from 73 to 100% (Fig. 1). Findings from this work suggest that these two emerging pathogenic *Chryseobacterium* strains may share similar protective antigens with *F. psychrophilum*. Furthermore, cross-protection to strain ARS202-14 is important as it appears to be a potential variant strain of *F. psychrophilum*. This strain exhibits external clinical signs and lesions atypical of most strains, grows rapidly at room temperature, and produces mortality in larger rainbow trout (>20g) following immersion challenge, a characteristic that to our knowledge has not been reported for other *F. psychrophilum* strains.

Figure 1. Cross-protection conferred by FP-B.17-ILM following immersion challenge with a putative variant strain of *F. psychrophilum* (ARS202-14), *C. oncorhynchi* and *C. piscicola*.



## IMPROVEMENTS IN PROTOCOLS FOR BROODSTOCK AND LARVAL REARING OF TRADITIONAL AND EMERGING SOUTHERN EUROPEAN MARINE FISH SPECIES

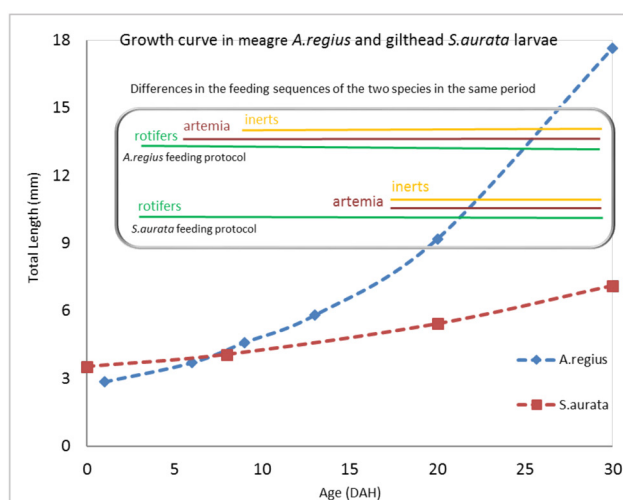
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Aquaculture Research Station (EPPO – Estação Piloto de Piscicultura de Olhão) located in the Southeast Coast of Portugal, in the confluence of the Mediterranean Sea with the Atlantic Ocean, is working with marine fish since 1984, i.e. the early start of modern marine aquaculture. Building on the large experience of producing gilthead seabream *Sparus aurata* and seabass *Dicentrarchus labrax*, we could adapt the protocols for stocking broodstock of several species of breams as red porgy *Pagrus pagrus*, white seabream *Diplodus sargus* and others species of the genus as *D. puntazzo*, *D. vulgaris* and *D. cervinus* and also sole, *Solea senegalensis*, aiming to diversify the Southern European aquaculture industry that is still highly depend on those *S. aurata* and *D. labrax*. Several groups of meagre *Argyrosomus regius* broodstock were established, initially wild specimens, while now own F1 have natural spawns during 3 or 4 months/year. We also reproduced and reared grouper, *Epinephelus marginatus* to adult stage for restocking. Different species requires different protocols and we could define the methodology for each, what allows to obtain good reliable quality spawns, with high hatching rate and without hormonal induction (except grouper). Our current emphasis is on zootechnical protocols and nutritional requirements for those species, to improve spawning, egg quality and larval and juvenile performance.

Meagre, is a strong candidate species for the expansion of the Mediterranean Aquaculture industry; with a fast growth, it achieves bigger sizes in a relatively short time, what makes this species propitious for filleting or other processing, adding value to the product, and making it more appealing to nowadays consumers. For meagre but also for gilthead seabream, seabass and sole, improvements can be achieved with different approaches, including improved live feed usage and special microdiets. In terms of nutrition optimal levels of essential nutrients are paramount, together with the use of premium ingredients and microencapsulation technologies. At EPPO facilities we have been working in larval rearing and testing diets to achieve the optimal levels of protein, the best lipid:protein ratio and phospholipids levels, by assessing their effects on fish quality, growth and survival.



**Acknowledgments:** Research funded by project DIVERSIAQUA (MAR2020, Portugal). Inert diets produced by SPAROS Lda.



## SHORT TERM APPROACH TO EPIDEMIOLOGY OF SKIN DISEASE IN THE NEW TARGET SPECIES FOR AQUACULTURE IN EUROPE *Holothuria arguinensis*

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Sea cucumber are seriously over-exploited by fishery pressure, pointing at aquaculture as profitable alternative for a highly demanding market. However, few studies have been performed on a common pathology known as skin ulceration disease, although it occurs worldwide on cultivated species. This study addresses epidemiological aspects of that disease in the new target species for aquaculture in Europe *Holothuria arguinensis*. Biomolecular techniques after one year survey across Ria Formosa Natural Park and Ramalhete Biological Station facilities, where sea cucumber aquaculture is developed, were applied to study epidemiology of this disease.

Each studied site showed a distinctive microbiota, although two shared genera were pointed as etiological agents: *Pseudoalteromonas* and *Vibrio*. An increase of prevalence was noticed from the second to the third trimester of year 2016 (Figure 1). An explanation could be attributed to a seasonal influence of environmental conditions on stress and immunological reactions. A long term population dynamic study leads to postulate an alternative hypothesis, which is related to a recorded increase of illegal captures that could have reduced healthier and larger individuals by fishery, and indirectly increased observed prevalence.

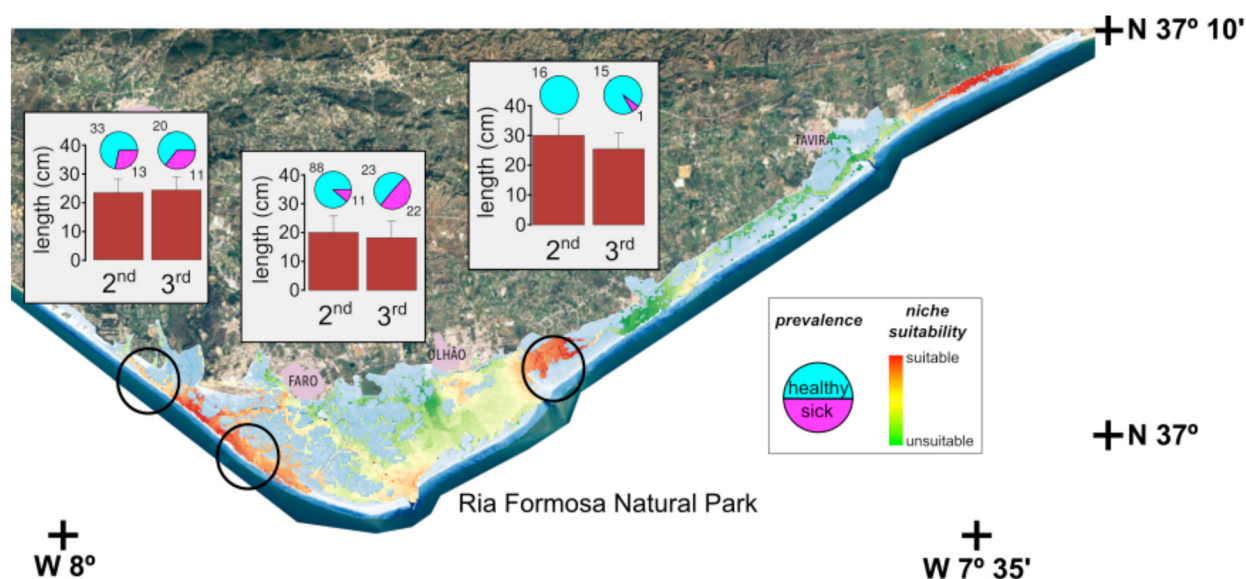


Figure 1.- Ecological niche calculated for *H. arguinensis* over the map of Ria Formosa Natural Park (see legend for color interpretation). Bar plots represent mean size and standard deviation for specimens collected for both 2<sup>nd</sup> and 3<sup>rd</sup> trimester in 2016 in Praia de Faro (east and west) and Armona locations. Piecharts show prevalence of skin ulceration disease with number of specimens indicated next to the plot (see legend for color interpretation).

## CONSECUTIVE EMAMECTIN BENZOATE AND DELTAMETHRIN TREATMENTS AFFECT THE EXPRESSIONS AND ACTIVITIES OF DETOXIFICATION ENZYMES IN THE RAINBOW TROUT

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*Caligus rogercresseyi*, an ectoparasitic copepod that negatively impacts the salmonid industry of southern Chile, is controlled through the application of emamectin benzoate (EMB) and/or deltamethrin (DM). The aims of this study were to determine the effects of three consecutive, alternating EMB and DM treatments on the protein and enzymatic activity levels of drug metabolizing enzymes, CYP1A, FMO GST, in liver, muscle, gills, kidney, and intestine of the rainbow trout (*Oncorhynchus mykiss*).

EMB/DM treatments resulted in a 10-fold increase and 3-fold decrease of CYP1A protein levels in the intestine and gills, respectively. CYP1A activity levels decreased in most of the analyzed tissues, reaching nearly 8- and 10-fold lower in the gills and intestine, respectively. FMO protein and activity levels increased in the kidney and, markedly, in the intestine. GST was up-regulated, with a notable 18-fold increase in gill GST protein levels. The enzyme activity levels of GST corroborated the up-regulated protein levels observed in all tissues. The altered activities of enzymes in EMB/DM treated rainbow trout could generate imbalances in detoxification processes, the synthesis and degradation of endogenous molecules with key biological activities, such as hormones, and complications associated with drug interactions for other treatments. It is especially important to keep this in mind when analyzing possible effects of consecutive treatments on salmon farming yields.

Effects of consecutive treatments with EMB and DM on the protein expression levels of CYP1A, FMO, and GST in different tissues of the rainbow trout.

|       | Liver      | Muscle      | Gills           | Kidney      | Intestine     |
|-------|------------|-------------|-----------------|-------------|---------------|
| CYP1A | 123 ± 22   | 115 ± 35    | 30 ± 27 *       | 127 ± 11 *  | 1,040 ± 344 * |
| FMO   | 245 ± 86 * | 72 ± 5      | 154 ± 51        | 631 ± 190 * | 735 ± 151 *   |
| GST   | 124 ± 13 * | 326 ± 167 * | 1,864 ± 556 *** | 112 ± 34    | 303 ± 87 *    |

Asterisks indicate significant differences between protein expression levels of treated relative to untreated rainbow trout samples (\*\*\* P < 0.001 and \* P < 0.05). Values are presented as the ratios ± SD of protein band densities (pixels) of treated samples compared to untreated samples, as computationally quantified from Western blot images.

Effects of consecutive treatments with EMB and DM on the enzymatic activity levels of CYP1A, FMO, and GST in different tissues of the rainbow trout.

|       | Liver        | Muscle      | Gills        | Kidney        | Intestine     |
|-------|--------------|-------------|--------------|---------------|---------------|
| CYP1A | 45 ± 22 *    | 268 ± 55    | 15 ± 1.1 *** | 56 ± 42       | 13 ± 4.3 ***  |
| FMO   | 129 ± 46     | 392 ± 119 * | 239 ± 146    | 796 ± 119 *** | 4,165 ± 459 * |
| GST   | 213 ± 13 *** | 412 ± 90    | 327 ± 142 *  | 365 ± 142     | 160 ± 86      |

Asterisks indicate significant differences between enzymatic activity levels of treated relative to untreated rainbow trout samples (\*\*\* P < 0.001 and \* P < 0.05). Values are presented as the ratios ± SD of activities measured for the enzymes of treated samples compared to those of corresponding untreated samples.



## **BUILDING HUMAN AND INSTITUTIONAL CAPACITY BY SUPPORTING HIGHER EDUCATION IN AFRICA**

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The development and strengthening of human capacity in partner countries is a central objective of the Feed the Future Innovation Lab for Collaborative Research on Aquaculture and Fisheries (AquaFish). AquaFish has developed an expansive collaborative network of international researchers and stakeholders dedicated to the sustainable development of aquaculture and fisheries. Providing support, mentorship, and academic guidance to students in post-secondary and graduate-degree programs in a wide array of disciplines helps early career scientists build and strengthen their professional networks. Between 2008 and 2015, over 400 students were supported by AquaFish in attaining post-secondary degrees, creating a pipeline of educated professionals who move on to careers in government, academia, and private enterprise upon graduation.

Human and institutional capacity has been built through the development of aquaculture, fisheries, and related curriculum and partnerships at many universities and centers for higher education in countries across the globe. In Ghana, AquaFish supported the establishment of advanced degree programs at Kwame Nkrumah University of Science and Technology (KNUST). To date, KNUST trained more than 400 Ghanaians and West African Nationals at undergraduate and graduate degree levels. In Kenya, the aquaculture facility at the University of Eldoret was established more than 15 years ago by AquaFish researchers from previous related projects through a series of short-term trainings for local fisheries department personnel, local farmers, and students. The ponds are still utilized today for training and research activities, resulting in a large network of individuals with knowledge of aquaculture best management practices.

Gender equality and women's empowerment in advanced education are core objectives of AquaFish. Therefore, a benchmark of at least 50% participation by women in long-term degree seeking programs was established to engage women throughout the sector, especially in research at universities and high level management in organizations. Between 2008 and 2015, 46% of the advanced degrees supported by AquaFish were earned by women and in 2016, 50% were women. Several of these graduates are now research partners involved in current projects, highlighting the benefits of early engagement and retention.

# GENETIC PARAMETERS FOR RESISTANCE TO ACUTE HEPATOPANCREATIC NECROSIS DISEASE, WHITE SPOT SYNDROME VIRUS AND SURVIVAL IN COMMERCIAL PONDS IN THE PACIFIC WHITE SHRIMP *Penaeus vannamei*

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Genetic parameters for survival to Acute Hepatopancreatic Necrosis Disease (AHPND) and to White Spot Syndrome Virus (WSSV) in challenge tests and three ponds; two commercial ponds with high mortality rates associated to these diseases and in pond with high biosecurity conditions. Shrimp come from 212 families yielded from a genetic growth line, a disease resistance line, and from several crosses between them. Families were identified by using color elastomers. Disease challenges were performed in laboratory controlled conditions in Sinaloa (Mexico). For AHPND challenge, shrimp were inoculated by immersion using  $1.4 \times 10^4$  CFU of *Vibrio parahaemolyticus* (strain M904), at an average age and body weight (standard deviation) of 83.1 (1.8) days and 1.4 (0.7) grams. For WSSV challenge shrimp were infected *per os* by using infected shrimp tissue with  $10^6$  viral particles at an average age and body weight of 92.7 (1.4) days and 2.3 (1.0) grams. Commercial ponds were located in Kino (Sonora) and Escuinapa (Sinaloa) in Mexico. Pond initial density was 14 shrimp/m<sup>2</sup> in both cases. The high biosecurity pond was also in Pozos (Sinaloa) and its initial density was 200 shrimp/m<sup>2</sup>. Shrimp were in the ponds from 65 to 130 days of age. Data were analyzed using a multivariate animal model and ASReml software. Genetic group was considered as a fixed effect, while body weight was considered as a covariate. Effects common to full-sibs was considered as a random effect. Survival rates were 51.4%, 9.4%, 26.6%, 14.2% y 87.0% for AHPND, WSSV, Kino, Escuinapa and Rosario. Although heritabilities were in general low, genetic correlations between survival in the disease challenge studies and the natural outbreak infected ponds were positive and from moderate to high. Hence, it is important to evaluate selection strategies using the experimental and natural outbreak results as the selection criteria in breeding programs for AHPND and WSSV endemic environments. Nevertheless, it is important to consider the existence of the observed negative genetic correlations observed between disease challenge studies and the pond where no infections occurred.

**Heritabilities (diagonal) and genetic correlations (above diagonal) for survival traits in the disease challenged populations, natural outbreak commercial ponds, and the high biosecurity pond.**

|           | NAHP             | SMB              | Kino             | Escuinapa        | Rosario          |
|-----------|------------------|------------------|------------------|------------------|------------------|
| NAHP      | <b>0.13±0.02</b> | 0.38±0.13        | 0.56±0.16        | 0.48±0.17        | -0.32±0.17       |
| SMB       |                  | <b>0.05±0.01</b> | 0.63±0.17        | 0.77±0.15        | -0.48±0.20       |
| Kino      |                  |                  | <b>0.06±0.03</b> | 0.92±0.12        | 0.51±0.72        |
| Escuinapa |                  |                  |                  | <b>0.06±0.03</b> | 0.42±0.31        |
| Rosario   |                  |                  |                  |                  | <b>0.05±0.03</b> |

## IMPROVED GENETICS AND A NOVEL FEEDING STRATEGY FOR IMPROVED GROWTH RATES OF NILE TILAPIA *Oreochromis niloticus* IN A RECIRCULATING AQUACULTURE SYSTEM

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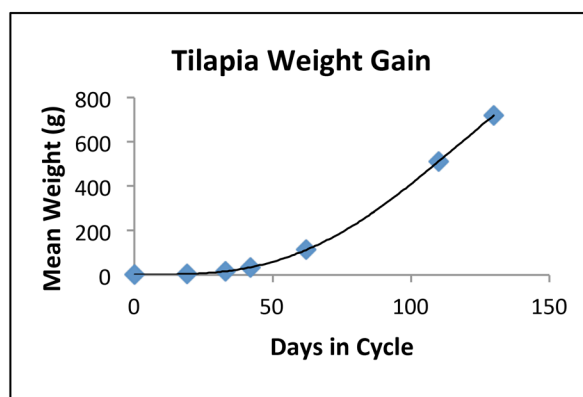
A Nile tilapia growth trial was conducted at the Pentair Aquatic Eco-Systems Inc. World Aquaculture Technology Engineering Research center (PAES W.A.T.E.R). The trial evaluated growth performance of a genetically improved strain of tilapia in a state-of-the-art recirculating aquaculture system (RAS). A robotic delivery system allowed for precision feed application and scheduling of daily feeding. A nutritionally complete feed with relatively high protein content (40%) was used for the duration of the trial.

The tilapia chosen for this trial were obtained from a breeder who has been selectively breeding their fish since 1988. The breeding program included a strain of the Genetically Improved Farmed Tilapia (GIFT) with partial origins from Africa. The supplier data suggested that it would take approximately 150 days to produce a 720 g fish.

A nursery system was stocked with 1.24 g fingerlings. After 42 days in the nursery system, 2,869 fish with a mean individual weight of 31.2 g were transferred to a single 32.5 m<sup>3</sup> grow-out tank capable of supporting a final density in excess of 60 kg/m<sup>3</sup>. Throughout the trial a robotic feeder was programed to feed the fish once every hour. Feed was adjusted weekly based off of calculated growth rates. Throughout the trial only minor interruptions in the feeding schedule occurred during periods of maintenance or during sampling events.

After a total of 130 days fish reached an individual harvest weight of 720 g. Final density was 61.8 kg/m<sup>3</sup> with an overall FCR of 1.2:1. Survival rate was 97.1%.

The improved growth performance was attributed to the regularity of the feeding regime. This suggests improved genetics in Nile tilapia along with an improved feeding schedule would reduce time required to reach market size. Additional production trials are planned to confirm results of this study.



# PRODUCTION OF MONOSEX NILE TILAPIA, *Oreochromis niloticus* BY DIETARY AND IMMERSION TREATMENT WITH *Basella alba* LEAVES AND *Tribulus terrestris* SEEDS

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Three days old mixed sex juveniles of Nile tilapia (mean weight  $0.025 \pm 0.009$  g) were subjected to dietary treatment with powdered *Basella alba* leaves and *Tribulus terrestris* seeds (0.0, 5.0, 10.0, 15.0 g/kg feed) and immersion treatment with aqueous extracts of both plant materials (0.05, 0.1, 0.15 g/l). There was no significant difference ( $P > 0.05$ ) in survival percentage among different treatment categories for both dietary and immersion experiments. Dietary treatment with both plant materials produced significantly higher percentage ( $P < 0.05$ ) of males compared to the control. There was no significant difference ( $P > 0.05$ ) in male percentage between treatments with *B. alba* and *T. terrestris* during feeding and immersion experiments. For dietary treatment, the highest percentage of males ( $76.6 \pm 0.5$ ) was observed with *T. terrestris* at the concentration of 15.0 g/kg (Table 1), while treatment with *T. terrestris* aqueous extract at a concentration of 0.15 g/l showed the highest percentage of males ( $81.4 \pm 0.5$ ) during immersion experiment (Table 2). However, further studies would be required to establish an ideal treatment regime for production of all-male tilapia population using the plant materials and to provide conclusive evidence regarding their efficacy to be used as a sex-reversal agent in tilapia culture.

TABLE 1: Percentage of survival, male, female and intersex during feeding treatment with powdered *T. terrestris* seeds at different concentrations. Different superscripts mark significant difference ( $P < 0.05$ ) in means within columns.

| Treatment category        | % survival       | % of male        | % of female      | % of intersex    |
|---------------------------|------------------|------------------|------------------|------------------|
| <i>Tribulus</i> 5.0 g/kg  | $97.5 \pm 2.5^a$ | $55.8 \pm 2.4^a$ | $26.2 \pm 3.6^b$ | $18.0 \pm 1.4^b$ |
| <i>Tribulus</i> 10.0 g/kg | $89.2 \pm 5.5^a$ | $64.1 \pm 0.8^b$ | $25.6 \pm 1.0^b$ | $10.3 \pm 1.5^a$ |
| <i>Tribulus</i> 15.0 g/kg | $84.2 \pm 6.8^a$ | $76.6 \pm 0.5^c$ | $14.2 \pm 2.0^a$ | $9.2 \pm 1.7^a$  |

TABLE 2: Percentage of survival, male, female and intersex during immersion treatment with aqueous seed extract of *T. terrestris* at different concentrations. Different superscripts mark significant difference ( $P < 0.05$ ) in means within columns.

| Treatment category       | % Survival       | % of male        | % of female         | % of intersex   |
|--------------------------|------------------|------------------|---------------------|-----------------|
| <i>Tribulus</i> 0.05 g/l | $83.3 \pm 1.7^a$ | $71.5 \pm 2.1^a$ | $21.3 \pm 3.2^{ab}$ | $7.2 \pm 1.1^a$ |
| <i>Tribulus</i> 0.1 g/l  | $80.8 \pm 2.2^a$ | $72.0 \pm 0.8^a$ | $23.0 \pm 0.5^b$    | $5.0 \pm 1.0^a$ |
| <i>Tribulus</i> 0.15 g/l | $81.7 \pm 1.7^a$ | $81.4 \pm 0.5^b$ | $13.4 \pm 0.7^a$    | $5.2 \pm 1.2^a$ |

# FISHERIES AND AQUACULTURE FUTURES IN AFRICA

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Fish, as a source of “rich food for poor people”, can play an important role in improving Africa’s food security and nutritional status. Despite the high dependence on fish as a source of animal protein, Africa has the lowest per capita fish consumption compared to other developing regions. At the same time, however, growth in fish consumption has been outstripping production. Aquaculture development in Africa has lagged far behind that seen in Asia. While developing countries as a whole are net fish exporters, the Africa region is a net fish importer. This paper examines past, present, and future trends of fish in Africa to explore policy implications and potential investment interventions to address the continent’s challenges in ensuring sustained fish growth to meet the growing regional demand.

Using the International Model for Policy Analysis of Agriculture Commodities and Trade (IMPACT) of the International Food Policy Research Institute (IFPRI) with an updated fish model developed in collaboration with WorldFish, the business-as-usual scenario of fish production, consumption, nutrient intake, and net trade in Africa region was projected out to 2050.

Growth in aquaculture production for Africa is projected to be low and likely to maintain a 2% share of the global fish output. Tilapia will continue to be the major species produced from aquaculture in the region. Aquaculture is expected to supply about a quarter of the fish consumed in Africa by 2030 while capture fisheries will continue dominance as a source of fish supply until 2050 (Figure 1). Although per capita fish consumption remains low, more imported fish is projected to meet demand from the growing population. Tremendous potential exists for aquaculture expansion in Africa, but will require important investments and will face considerable challenges.

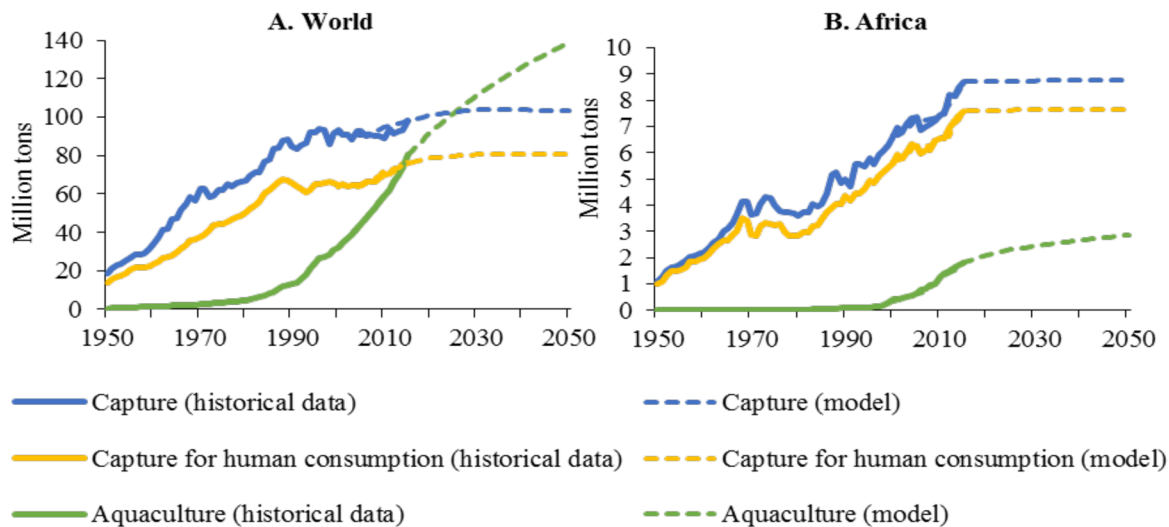


Figure 1. World (A) and Africa (B) capture fisheries and aquaculture production, 1950-2050

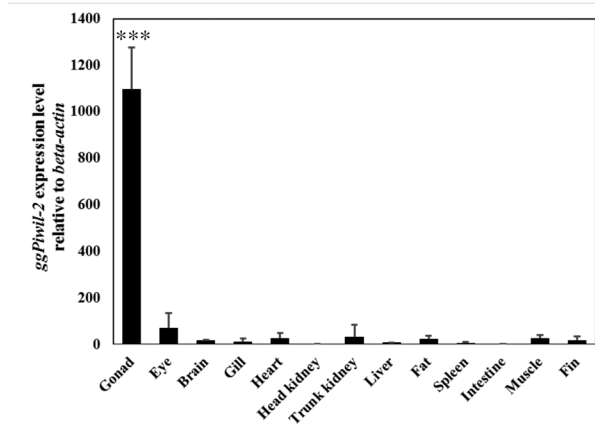
## CHARACTERIZATION OF PRIMORDIAL GERM CELLS IN GIANT GROUPER BY PIWIL2 AS GONAD SPECIFIC MARKER

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Groupers are famous for being a remunerative species of aquaculture in Asia. One of the bottlenecks in giant grouper breeding is their long-term sex maturation which takes as many as 7 to 10 years. The groupers of genus *Epinephelus* is a protogynous teleost that they only perform sex transformation until a specific body size is reached. To elucidate the gonad formation and sex development process, gonad specific expressed gene, Piwil-2, is cloned for monitoring the germ cells.

To characterize the gonad development of grouper, Piwil-2 is cloned from giant grouper, *Epinephelus lanceolatus*, called ggPiwil-2. ggPiwil-2, containing conserved PAZ and PIWI domain, is involved in the piRNA pathway related to germ cell survival and differentiation. From the qRT-PCR results, the genes is specifically expressed in gonad while other somatic tissues only show low levels of ggPiwil-2. For further protein level detection, anti-ggPiwil-2 antibody is produced in-house. Only in gonad tissue can ggPiwil-2 protein be detected which parallel to the gene expression pattern. We have as well collected grouper larva during early development stage, and found that primordial germ cells are able to be identified with ggPiwil-2 antibodies by whole-mount immunofluorescence. These data have shown that the molecular markers, ggPiwil-2, is a potential research tool for gonad development research.



**FIGURE 1. Tissue distribution of ggPiwil-2 in giant grouper. Expression was measured by qRT-PCR and normalized to  $\beta$ -actin. The tissue with lowest expression was set as 1 for comparison. Results represent means and standard of six individual fishes. Values were compared using the t-test (\*\*\*) $p < 0.001$ .**

# LARVAL REARING OF THE PACIFIC WHITE SHRIMP IN ZERO WATER EXCHANGE SYSTEM

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The objective of the study is to establish zero water exchange larval culture technology in *Litopenaeus vannamei* to produce more than 100 PL3 in one liter of water. Larvae were reared in 500 and 2,000 liters FRP tanks, three stocking density (100,150, 200 nauplius/L) with 2 replicates were tested in each experiment. Test results are as follows: In 500 liter tanks, the average survival rates were  $45.7\% \pm 1.0\%$ ,  $47.3\% \pm 0.5\%$ ,  $47.9\% \pm 0.3\%$ , and the average yield were  $45.7 \pm 1.0$ ,  $94.7 \pm 1.0$ ,  $143.8 \pm 0.8$  of PL3/L, respectively. In 2,000 liter tanks, the average survival rates were  $67.7\% \pm 3.6\%$ ,  $63.0\% \pm 3.3\%$ ,  $50.1\% \pm 0.9\%$ , and the average yield were  $67.7 \pm 3.6$ ,  $126.0 \pm 6.6$ ,  $150.3 \pm 2.8$  of PL3/L, respectively.

Table1. The survival and yield of larval rearing of the pacific white shrimp in 500 liter tanks in zero exchange system.

| Nauplius (#) | stocking density (nauplius/L) | PL3 (#) | Survival (%) | Yield (PL3/L) | Average survival (%) | Average Yield (PL3/L) |
|--------------|-------------------------------|---------|--------------|---------------|----------------------|-----------------------|
| 50,000       | 100                           | 22,500  | 45.0%        | 4.5           |                      |                       |
| 50,000       | 100                           | 23,200  | 46.4%        | 4.6           | $45.7 \pm 1.0\%$     | $45.7 \pm 1.0$ ,      |
| 100,000      | 150                           | 47,700  | 47.7%        | 9.5           |                      |                       |
| 100,000      | 150                           | 46,980  | 47.0%        | 9.4           | $47.3 \pm 0.5\%$     | $94.7 \pm 1.0$ ,      |
| 150,000      | 200                           | 71,640  | 47.8%        | 14.3          |                      |                       |
| 150,000      | 200                           | 72,180  | 48.1%        | 14.4          | $47.9 \pm 0.3\%$     | $143.8 \pm 0.8$       |

Table 2. The survival and yield of larval rearing of the pacific white shrimp in 2,000 liter tanks in zero exchange system.

| Nauplius (#) | stocking density (nauplius/L) | PL3 (#) | Survival (%) | Yield (PL3/L) | Average survival (%) | Average Yield (PL3/L) |
|--------------|-------------------------------|---------|--------------|---------------|----------------------|-----------------------|
| 200,000      | 100                           | 140,400 | 70.2%        | 7.0           |                      |                       |
| 200,000      | 100                           | 130,320 | 65.2%        | 6.5           | $67.7 \pm 3.6\%$     | $67.7 \pm 3.6$ ,      |
| 400,000      | 150                           | 242,640 | 60.7%        | 12.1          |                      |                       |
| 400,000      | 150                           | 261,360 | 65.3%        | 13.1          | $63.0 \pm 3.3\%$ ,   | $126.0 \pm 6.6$ ,     |
| 600,000      | 200                           | 304,560 | 50.8%        | 15.2          |                      |                       |
| 600,000      | 200                           | 296,640 | 49.4%        | 14.8          | $50.1 \pm 0.9\%$     | $150.3 \pm 2.8$       |



# DETECTION AND MOLECULAR CHARACTERIZATION OF RANAVIRUSES DETECTED IN NILE TILAPIA (*Oreochromis niloticus*) IN TANZANIA

Augustino Chengula\*, Christopher Kasanga, Stephen Mutoloki, Robinson Mdegela and Øystein Evensen

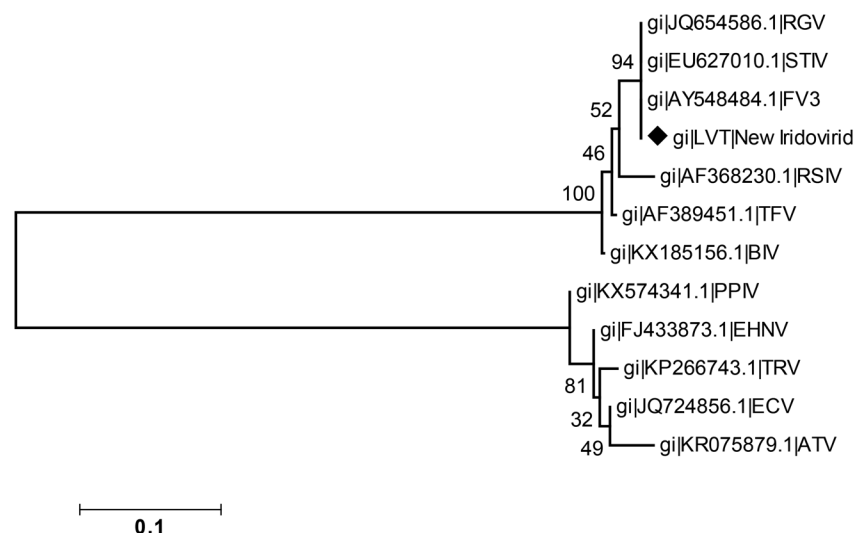
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Ranaviruses are significant pathogens infecting all classes of ectothermic vertebrates (fish, reptiles, and amphibians) causing systemic necrotizing infections and mass mortalities in captive and wild populations across the globe. Ranaviruses are large DNA iridovirids belonging to the family *Iridoviridae*. There many published reports of ranaviruses infecting different fish species worldwide from both fresh- and marine water with clinical manifestations ranging from subclinical to death. However, based on our understanding there no reports of ranaviruses infecting Nile Tilapia in Tanzania. Therefore, the aim of this study was to identify and characterize ranaviruses infecting Nile Tilapia in selected natural water bodies and fish farms in Tanzania. Samples (Nile Tilapia) were collected from Lake Victoria (108 fish) in Mwanza and fish farms (166 fish) in Morogoro, Tanzania. Fish were examined grossly, euthanized, dissected, examined internally and organs of interest collected. Organs collected included spleen, kidney, liver, heart, gills, fins and skin. Organs were homogenized using mortar and pestle, then stored at -80°C for further analysis.

A conventional PCR was performed targeting viral DNA polymerase and PCR products sequenced. Screening of remaining samples was performed using real time PCR targeting helicase gene. The main clinical and pathological findings included haemorrhages on the ventral part of the body, liver and at the base of the pectoral fins, unilateral corneal opacity and exophthalmia, enlargement of the spleen and liver, mushy liver, whitish mottling on the surface, congested blood vessels and ecchymotic haemorrhages on the liver. Our findings show that wild Nile tilapia in Tanzania contain Iridovirids homology with *Rana grylio virus* (96%), *Soft-shelled turtle iridovirus* (96%) and *Frog virus 3* (95%) and they cluster together in the phylogeny (Figure 1). These findings suggest that the isolates belong to the genus *ranavirus*. These findings complement the previous hypotheses of the possible host-switch of ranaviruses from one host to another and vice versa.

Based on our knowledge, this is the first report of molecular characterization of ranaviruses in Nile Tilapia from Tanzania.

**Figure 1:**  
Phylogenetic analysis of partial  
DNA polymerase sequences



## WCONTRIBUTION OF SMALL-SCALE AQUACULTURE TO RURAL LIVELIHOODS IN TANZANIA

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Aquaculture is one of the world's fastest growing animal producing sector with an average growth rate of 8.8% outpacing capture fisheries (1.2%) and terrestrial farmed meat production (2.8%). Globally, aquaculture is considered as an important sector for poverty alleviation and rural development. In Tanzania aquaculture is primarily based on subsistence farming of pond cultured Nile tilapia (*Oreochromis niloticus*). In recent years there has been increased interest on promoting aquaculture as a tool for poverty reduction and combating malnutrition in rural areas. Many non-governmental organizations, church based organizations and research institutes/Universities have been distributing Nile tilapia fingerlings to small-scale farmers in rural areas as an effort to contribute effectively to poverty alleviation and improve food security among the rural poor people. Currently it is estimated that there are 20,235 fish ponds owned by 17,725 small-scale farmers. Most fish farmers in the country prefer to produce Nile tilapia (*Oreochromis niloticus*) and few culture African catfish (*Clarias gariepinus*). The main objective of this study was to determine the contribution of aquaculture enterprise to income and wellbeing of rural households in Tanzania. A household survey was carried out in four districts (Morogoro Rural, Kilosa, Mpwapwa and Mufindi districts) in Tanzania. A total of 60 small-scale fish farmers (15 per district) were randomly selected and individually interviewed. The study found that, on average farmers owned  $2.8 \pm 0.6$  ponds which had a mean ( $\pm$  se) size of  $258.5 \pm 74.0$  m<sup>2</sup>. All farmers cultured Nile tilapia and the main reasons for engaging in fish farming were production of fish for home consumption and generation of household income. On average, 23.6% of the fish harvested were consumed by the family while the remaining 76.4% were sold. Most households consumed fish either two to three times in a month (36.7%) or once per month (31.7%). All farmers reported that they consume fish which are harvested from their own ponds and rarely they buy from the village markets. All fish farmers sold fresh fish directly to consumers (75%), fish vendors (35%) and retailers (20%). The mean ( $\pm$  se) annual income from fish farming was TZS 826,357.1  $\pm$  179,764.5, and this contributed 19.3% of the total household income. The income from aquaculture enterprise was used for house construction (45%), to pay school fees (40%), buy consumer goods (25%), buy livestock to increase herd size (15%), pay medical bills (5%), pay costs for crop farming (5%) and buy food during period of food shortage (5%). The study revealed that small-scale aquaculture enterprise contributes significantly to household income and wellbeing of rural farmers.

## THE IMPLICATIONS TO AQUACULTURE OF *Mugil cephalus* DIVERSITY IN TAIWAN WATERS

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*Mugil cephalus* is an important aquaculture species in Taiwan because of its highly valuable roe. In order to produce large numbers of roe of an adequate size, the mullet fry are captured from the wild and then raised in aquaculture ponds until maturity. However, not all mature females develop ovaries and some only develop small ovaries. Thus, it seems likely that genetics plays an important role in how the various mullet populations found in Taiwan waters mature and produce such a wide variety of roe sizes under identical aquaculture conditions. The aim of the study is the identification of useful genetic markers for selecting mullet that produce large roe under aquaculture conditions. The correlation of ovary size, body size and gonadosomatic index (GSI) with each mtDNA profile of the cultured mullet has been investigated in detail. The presence of further hidden genetic structure that could help to explain the differences of roe size across mullet populations around Taiwan is also explored by using microsatellites.

Three cryptic species of *Mugil cephalus* (NWP1, NWP2 and NWP3), identified using the multiplex cytochrome oxidase subunit I (CO I) haplotype-specific PCR, were cultured in aquaculture ponds. The NWP1 females generally have the largest body size, but fail to have developed ovaries by 3 years of age. After an additional year of culture, the NWP1 females were found to yield only small ovaries. By way of contrast, the NWP2 females, which have the smallest body size, generally yield larger ovaries with the size of the roe depending on the age of the fish, 2, 3 or 4 years old. However, within this mitotype, most of the eastern NWP2 population yield only small ovaries, which suggests further subdivision of these mitotype based subspecies. The rarest mitotype, NWP3, produce significantly larger ovaries, as well as a bigger body size, at 3-years old than 3-year-old NWP2 females. Thus, both NWP2 and NWP3 fry can be considered to be suitable subjects for mullet aquaculture. On the other hand, NWP1 fry are not suitable for aquaculture on economic reasons and should be left as a fishery resource in the sea if possible.

The nuclear population genetic structure has also been analyzed using ten microsatellite loci. The sampled Taiwanese populations, whether classified temporally or geographically, were found to be highly dynamic and genetic differentiation into sub-populations at a nuclear DNA levels was found, in addition to the above differentiation on mitotype. The findings suggest the presence of at least five subpopulations within the coastal waters of Taiwan, which agrees with anecdotal evidence from local fishermen. These genetics of these subpopulations are also likely to have an effect on ovary and body size which is likely to affect the choices made by Taiwanese fish farmers.

## EXTENSION OF SMALL-SCALE TILAPIA FARMING IN MADAGASCAR

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In Madagascar, aquaculture of Nile tilapia (*Oreochromis niloticus*) has not yet been well developed despite its early introduction from the African Continent to the country in 1956. Nowadays, in Boeny Region, situated in the Northwestern side of the island, the species is very common for rural folks to capture in the natural water bodies and to consume both at home and restaurants. It would be a potential commodity to generate additional income to small producers and to provide animal protein to those rural families who suffer nutrient deficiency. The Ministry of Fisheries Resources and Fisheries (MRHP), Madagascar implemented a tilapia culture-based rural development project in cooperation with Japan International Cooperation Agency (JICA) from April 2011 to February 2016. As a result of the project interventions, a total of 1,500 farmers were trained, of which an estimated number of 500 farmers (33%) actually practice tilapia farming. The Project has contributed to an increase in the number of tilapia farmers from 34 in April 2011 to 500 (14.7 times more) in February 2016 in three target districts.

The Project adopted so-called farmer-to-farmer (FTF) extension approach. In the absence of adequate extension system, it was expected for farmers to play a pivotal role of the extension. The Project selected a group of progressive farmers and trained them as seed producers. Then, they were encouraged to become core-fish farmers (CFFs) to perform as extension agents or service providers. They produce and supply healthy tilapia seed and at the same time transfer grow-out technology to other fellow farmers. As long as those CFFs benefit economically and socially from the extension works, the FTF extension system will be sustained even on an individual basis.

Furthermore, networking of the CFFs was instrumental for securing sustainability of the farmer-based extension system. The CFFs themselves recognized needs and advantages of the networking since they actually enjoyed benefits from the cooperation each other. For example, technical exchange and transfer among CFFs made it easy to improve techniques on broodstock management, breeding, fry nursing and rearing. They also practiced lending, buying, selling or accommodating breeders, equipment/materials and other inputs to minimize production costs. Proper coordination and cooperation in the marketing was made possible through the network to maximize profit making opportunities. Joint procurement of production inputs often resulted in cost reduction. The network is a sort of farmers' loose organization which has better access to government offices than individuals. It is a group of CFFs and each CFF has a group of grow-out farmers as customers for tilapia seed. Therefore, as a whole, the network can represent all the farmers concerned with tilapia culture, and it obviously demonstrates rural empowerment. It is considered the tilapia farming can be sustained as long as the network is maintained functional.

# LOW SALINITY ACCLIMATION IN HYBRID BETWEEN RED-SPOTTED GROUPER *Epinephelus akaara* AND GIANT GROUPER *E. lanceolatus* AND GENE ENCODING PROLACTIN

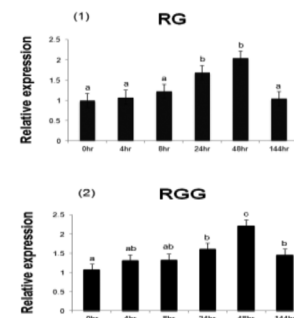
Bok-Ki Choi\*, YeoReum Kim, Han Kyu Lim, Jong-Myoung Kim

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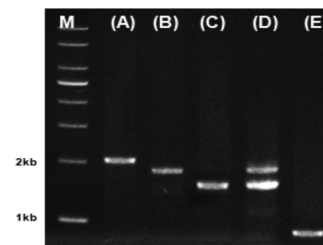
In fish living in aquatic environment, environmental salinity affects various processes such as growth, development, and biorhythm of the host. Prolactin (PRL) has been known to play a key role in osmoregulation in fish. In order to understand its roles in osmoregulatory mechanism and growth performance under different salinities in fish, the gene encoding PRL and its expression level upon acclimation to low salinities were investigated in two species of grouper, red-spotted grouper (RG) *Epinephelus akaara* and its hybrid red-giant grouper (RGG) generated from *E. akaara* ♀ and *E. lanceolatus* ♂. Survival rates of groupers under different salinities at 32, 24, 16 and 8 psu indicated that RGG survived at salinities as low as 8 psu while all RG died at 8 psu. Significant differences in body weight and total length were observed at the end of experiment in RG and RGG. The result also indicated a higher rate of growth observed at the 16 psu as compared to that of 32 psu. In order to examine the mechanism associated with osmoregulation, the genes encoding prolactin were obtained from genomic DNA and brain cDNA templates prepared from RG and RGG. PRL genes in RG and RGG consist of five exons encoding the open reading frame of 212 amino acid including 24 amino acids of signal peptide and mature protein of 188 amino acids.

To examine expression pattern of PRL upon acclimation to low salinity in RG and RGG, RNAs were isolated from brain (include pituitary) of RG and RGG collected at 0, 4, 8, 24, 48, and 144 hrs after decreasing the water salinity. Amplification of cDNA was carried out by using primers specific to PRL and beta-actin gene as a positive control. Expression of PRL were increased during 48 hrs upon acclimation to low salinity and then decreased (Figure 1.) in both RG and RGG. This result suggests the osmoregulatory role of PRL in RG and RGG.

Sizes of exons in cDNA sequence and structure of PRL were similar in RG and RGG although the sizes of introns were different. Differences in the sizes of introns in RG and RGG can be applicable to develop a method for identifying the specific grouper species (Figure 2).



**Figure 1.** Expression of PRL in RG (1) and RGG (2) upon acclimation to a low salinity.



**Figure 2.** Development of a species-specific detection method for groupers based on PRL sequences. M: DNA ladder, (A) Kelp grouper, (B) Giant grouper, (C) Red-spotted grouper, (D) Hybrid grouper, (E) Seven-band grouper.

EFFECTS OF RECOMBINANT VERTEBRATE ANCIENT LONG OPSIN INJECTION ON REPRODUCTION IN GOLDFISH, *Carassius auratus*: PROFILE OF THE GREEN WAVELENGTH LIGHT

Ji Yong Choi, Cheol Young Choi\*

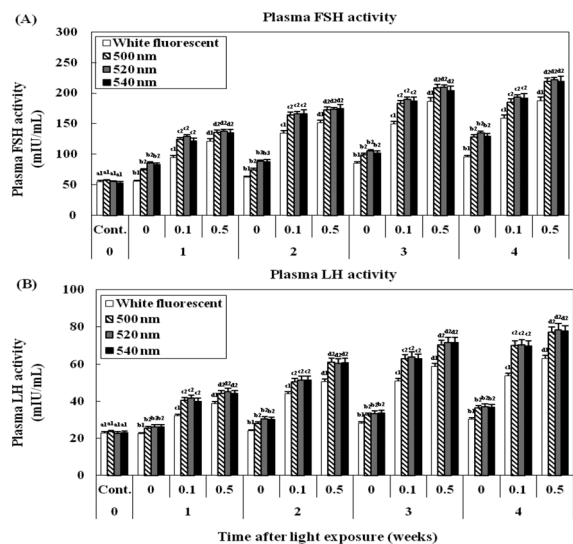
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This study was conducted to identify the possible effect of recombinant vertebrate in ancient long (VAL) opsin as non-visual “photoreceptor” in deep brain on the reproduction of goldfish, *Carassius auratus*. In addition, we also investigated the effects of the green wavelength light on the predictable reproductive function of VAL-opsin as green sensitive pigment in deep brain. In order to comply with the purpose, we measured the changes in the expression levels of GTHs (GTH $\alpha$ , LH $\beta$ , and FSH $\beta$ ) and estrogen receptor (ER: ER $\alpha$  and ER $\beta$ ) mRNAs associated with the reproduction of goldfish. As well as the changes in plasma follicle stimulating hormone (FSH), luteinizing hormone (LH) and 17 $\beta$ -estradiol (E $_2$ ) activities, after injection of recombinant VAL-opsin protein in the two concentration (0.1 and 0.5  $\mu$ g/g body mass) for 4 weeks (injection once a week), and also examined the possible impact of green wavelength lights (500, 520, and 540 nm) on the function of the VAL-opsin. As a result, all parameters associated with reproduction were significantly increased as time with the passage of time and the exposure to LED. Through the results of this study, we can suggest that the VAL-opsin existed in deep brain is involved in the maturation of goldfish, in addition, and it is expected that the green wavelength region serve to improve the ability of VAL-opsin by increasing the val-opsin expression.

Acknowledgment

This research was supported by the project titled ‘Development of the eco-friendly copper alloy net for antifouling and the fish farming cage’ funded by the Ministry of Oceans and Fisheries.

Figure 1. Changes in the activities of plasma FSH (A) and LH (B) in goldfish under three green wavelength lights and a white fluorescent bulb following VAL-opsin (0.1 and 0.5  $\mu$ g/g BM) injection. The experimental groups were exposed to green wavelength region (500, 520, and 540 nm) LEDs; the light intensity at the water surface was approximately 0.5 W/m $^2$ . Different letters indicate significant differences among treatments exposed to different LED spectra at the same time ( $P < 0.05$ ). Different numbers indicate significant differences among time points for the same LED spectra exposure ( $P < 0.05$ ). All values are means  $\pm$  SD ( $n = 5$ ).



## EFFECTS OF WATERBORNE COPPER ON OXIDATIVE STRESS AND IMMUNE RESPONSES IN RED SEABREAM, *Pagrus major*

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Copper (Cu) plays an essential role in many enzymatic processes including cellular respiration. In this study, we investigated the oxidative stress and immunity of red seabream, *Pagrus major*, exposed to different concentrations of Cu (10, 20, 30, and 40  $\mu\text{g/L}$ ) for 120 h. We measured the expressions of mRNA, activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), the levels of plasma hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and immune parameters lysozyme, immunoglobulin M (IgM), and melatonin. The mRNA expressions, activities of antioxidant enzymes, and the levels of plasma  $\text{H}_2\text{O}_2$  were significantly higher after exposure to 30 and 40  $\mu\text{g/L}$  of Cu than after exposure to lower concentrations (0, 10, and 20  $\mu\text{g/L}$ ). However, the levels of plasma lysozyme, IgM, and melatonin were significantly lower after exposure to 30 and 40  $\mu\text{g/L}$  of Cu. These results indicate that Cu concentrations of 30 and 40  $\mu\text{g/L}$  can induce an acute toxic oxidative stress and decrease the immune response in red seabream in aquarium conditions.

### Acknowledgment

This research was supported by the project titled 'Development of the eco-friendly copper alloy net for antifouling and the fish farming cage' funded by the Ministry of Oceans and Fisheries, Korea

**Figure 1.** Comet assay images (A) and comet assay parameters (B) tail length and percentage DNA in tail under different concentrations of Cu [0 (Cont.), 20 and 30  $\mu\text{g/L}$ ] for 0 and 120 h. White arrows (in A) indicate damaged nuclear DNA (DNA breaks) of liver cells which are stained with SYBR-green. Scale bars = 100  $\mu\text{m}$ . The lowercase letters (in B) with different characters indicate significant differences between different concentrations at the same time ( $P < 0.05$ ). The asterisk (\*) indicates significant differences between different times within the same concentration ( $P < 0.05$ ). All values are means  $\pm$  SE ( $n = 5$ ).

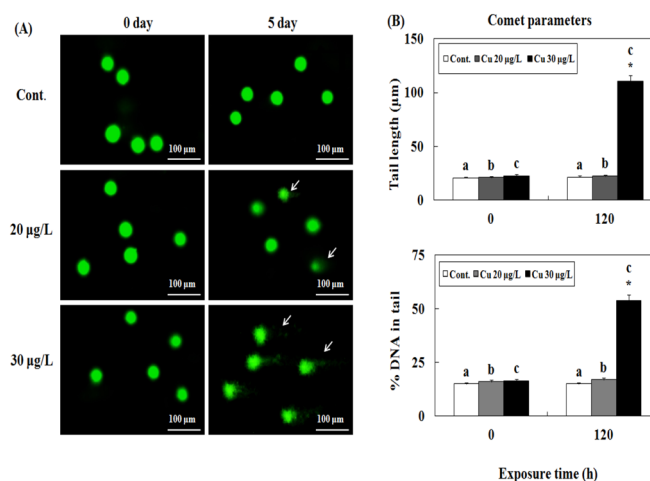


Figure 1.



INTESTINAL MICROBIOTA IN PACIFIC ABALONE *Haliotis discus hannai*

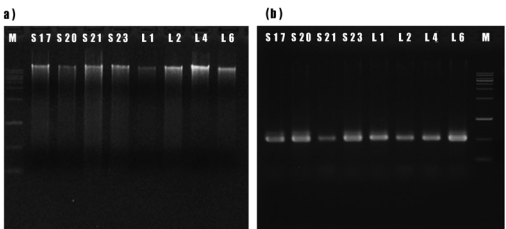
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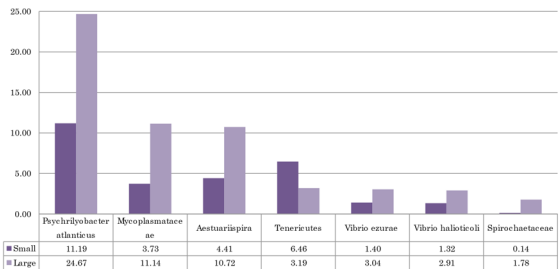
Microbial community in the intestine of marine organisms may affect the host physiological and metabolic statues including food digestion, nutritional metabolism and immunity against invasive pathogens and in an opposite way. Therefore, understanding the interaction between host and intestinal microbial community is important for aquaculture industry to enhance the productivity. In this study, differential intestinal microbial composition between large and small pacific abalones was analyzed by using 454-pyrosequencing method as a culture-independent analysis. Culture-dependent analyses were also carried out by using alginate-enriched media and by its digestive ability to degrade alginate.

To compare the microbial composition in the intestines of slow and fast growing abalones, two size (Small and Large) groups of abalones of 600 dpf (days post fertilization) were collected from southern part sea of South Korea. Abalones of approximately 3-fold differences in weighs between two groups were subjected to the study. Intestines dissected from each abalone were subjected for isolation of metagenomic DNA. Quantity and quality of DNA was assessed by nanodrop and 16S rDNA PCR (Figure 1). Metagenomic DNA of eight individual abalones from each group were analyzed by 454-pyrosequencing.

Out of valid reads of 6970, average 876 operational taxonomic units (OTUs) were obtained of which the value is higher in a small group (968.25) than that in a large group (510.25) indicating a higher microbial diversity in a small size abalone. One of the dominant species found in a large group is *Psychrilyobacter atlanticus* that has the metabolic capacities to utilize glucose, fructose, citrate, and pyruvate as a carbon source. Species composition showing a high difference between two groups were selected as illustrated in Figure 2. (*P. atlanticus*, *Mycoplasmataceae*, *Tenericutes*, and *Aestuariaispira*). *P. atlanticus* accounts for more than 50% population in a large group (Figure 2). For the culture dependent analysis, marine broth containing 0.5% sodium alginate was used and isolated culture were identified by 16s rDNA sequencing. The digestive enzyme activity of each culture would be discussed.



**Figure 1.** (a) Metagenomic DNA isolated from 8 intestines of 600 dpf *H. discus hannai*. (b) PCR product amplified using same amount of metagenomic DNA as template and 16S rDNA universal primers (27F/518R).



**Figure 2.** Numbers account for the percentages of microorganisms in the intestine of small and large size abalones.

## CLONING AND ANALYSIS OF CALMODULIN-LIKE PROTEIN GENES IN PACIFIC ABALONE, *Haliotis discus hannai*

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Calcium ion plays an important role in many cellular processes, such as muscle contraction, neuronal transmission, cellular motility, cell growth and proliferation through the reaction with calcium binding proteins. Calmodulin like (CaML) related to the calmodulin family of calcium binding proteins play a role in cellular calcium ion-dependent signal pathways.

In the previous study, we identified four canonical calmodulin genes with amino acids similarity of above 92% in Pacific abalone (*Haliotis discus hannai*), called CaM A, CaM B, CaM C, and CaM D. In this study, we additionally identified three calmodulin like genes (CaML) with amino acids similarities of 60%-85% to canonical calmodulin, designated CaML A, CaML B, and CaML C.

The open reading frames (ORFs) of the CaML A and CaML B genes were 471 and 459 nucleotides, encoding 157 and 153 amino acid residues, respectively. The CaML C gene encodes two other types of RNA, CaML C-type1 and CaML C-type2. The ORF of CaML C-type1 and CaML C-type2 both were 447 nucleotides, encoding 149 amino acid residues. Their identities among nucleotide of four ORFs are 60 % to 75 %, whereas the 5'- and 3'-UTR are highly variable. Similar to the previously studied ORF of CaM A, the identities of nucleotide were confirmed to be 60% to 85%. In the case of the amino acid sequence, it was confirmed that their identities were 60% to 85% as compared with CaM A (Figure 1).

As a result of comparing cDNA and genomic DNA sequences, it was confirmed that CaML A and CaML B genes consisted of 5 exons and 4 introns. However, we found that the CaML C gene encodes 7 exons and 6 introns. CaML C gene are alternatively spliced, CaML C-type 1 RNA is spliced with C-1-E4 and E5, and CaML C-type2 RNA is spliced with C-2-E4 and E5. (Figure 2).

In this study, the sequence of CaML gene derived from *H. discus hannai* was identified, and the similarity of CaML cDNA and amino acid sequence with CaM gene was confirmed. However, it has not been confirmed how these CaML genes are expressed in each tissue. In the next study, we will perform a study on the expression level of CaML genes in each tissue.



Figure 1. Amino acids sequence alignment of CaML genes.

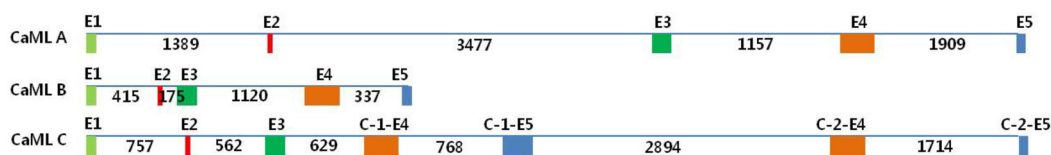


Figure 2. The genomic structures of CaML genes.

## CONDUCTIVE AQUACULTURE TECHNOLOGIES FOR WOMEN: POTENTIAL AND IMPACTS

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In its efforts to explore opportunities to involve more women in aquaculture and unravel the barriers that women face in successfully uptaking the practices that could lead to better income and nutritional outcomes, WorldFish initiated research on fishing gears that women could more easily use. In many of its efforts to involve women, WorldFish has been struggling with enabling women to take more control over aquaculture production and in receiving the benefits from it. Even when women are trained in aquaculture for their homestead system over which they have more control and provided with access to inputs, it has been found that women do not find it easy to take up many of the roles that the technology demands. They in fact remain reliant on the men to perform many of the activities, for reasons ranging from social norms around what roles they are and are not supposed to or able to perform to the inability of the technology to suit the women's needs and abilities. In fact, in a CCAFS and AAS study on gender relations and technology adoption, it has been found that perceptions around women's ability to perform the required tasks due to limitations in knowledge or physical strength may enable men to maintain control over technology use.

To make harvesting of nutrient-rich mola accessible to women from within the homestead pond, the project developed a new harvesting technique for ponds with a gill net consisting of two mesh sizes that can catch mola fish of various sizes and without having to enter the pond and getting wet. The net takes about 10 to 30 minutes to set depending on the pond conditions, 1-2 hours for submersion and another 10-15 minutes to harvest. The submersion time allows women to finish off other errands. A gill net can be used every day at little cost. The women have found it especially convenient as they do not have to wait for their husband's to come home and catch fish for them or purchase fish. A single harvest of mola, depending on season and stock, can be enough for one family meal but women have reported that they value even a few pieces being caught, as they can feed it to their children.

A study has been conducted with users of the gill net to understand its consumption impacts and the involvement of women. Weekly data collected over a six month period by AIN enumerators has shown that the average frequency of netting went up from 1.2 times per week to 3.4 times per week. Gill net users ate mola on average 3.1 times per week while non-gill net users ate mola 1.2 times per week, showing that besides making harvesting accessible to women, the new technology is boosting household consumption of a nutrient rich fish. Finally social consciousness raising exercises have been conducted with family and community members to build women's acceptance in the usage of this net. Before and after empowerment surveys with the women and attitude surveys with the communities have shown promising results.

## DIETARY PROTEASE IMPROVES NUTRIENT AND ENERGY RETENTION EFFICIENCY IN CHINESE MITTEN CRAB *Eriocheir chinensis*, FED LOW FISH MEAL DIETS IN LABORATORY CONDITIONS

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A 12-week trial was conducted to investigate growth performance, nutrient and energy retention in Chinese mitten crab *Eriocheir chinensis* fed a high fishmeal diet (positive control) and four low fishmeal diets supplemented with graded level (0, 125, 150 and 175 mg kg<sup>-1</sup>) of a dietary protease (Jefo Nutrition Inc., Canada) at Suchow University, China. All five diets were formulated to be isoproteic (40.4 ±0.3% CP) and isoenergetic (18.6 ±0.2 kJ g<sup>-1</sup>). No differences were observed among the treatments in weight gain (g), specific growth rate (%), survival (%), protein and lipid deposition (PD, g crab<sup>-1</sup> and LD, g crab<sup>-1</sup>), recovered energy (RE, kJ crab<sup>-1</sup>), and energy retention efficiency (ERE, %). Significant differences were observed in protein and lipid retention efficiencies (PRE and LRE, %) among the treatments, where LRE was the highest (37.9%) in crabs fed diets supplemented with 175 mg kg<sup>-1</sup> protease followed by those fed the positive control diet. A linear regression of the parameters under negative control diets showed significant effects of the level of protease on hepatopancreatic protease activity ( $P=0.001$ ), nutrient retention efficiencies, PRE ( $P=0.05$ ) and LRE ( $P=0.014$ ) but not on ERE (Table 1). The principal component analysis (Figure 1) showed that nutrient and energy retention efficiency and hepatopancreatic protease activity (HPRO) are positively correlated and FCR was negatively correlated ( $P<0.05$ ) with the dietary protease. On the other hand, the growth parameters such as weight gain was significantly correlated with PD, LD and RE.

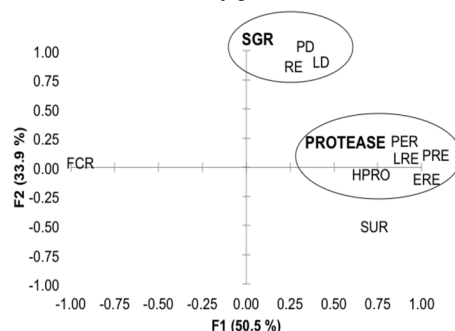
This study is one of the first to show that dietary enzymes and in this case, a commercial protease drives the nutrient and retention efficiencies in farmed animal and thus, highly useful for better utilization of dietary nutrients.

Table 1: Protein and lipid retention efficiency (PRE and LRE) and hepatopancreatic protease activity (HPRO) in Chinese mitten crab fed diets with and without protease

| Dietary treatments | PRE, %        | LRE, %       | HPRO       |
|--------------------|---------------|--------------|------------|
| PC                 | 30.0 ±3.2% ab | 35.0 ±3.3%a  | 4480 ±288b |
| NC                 | 25.0 ±3.2%b   | 27.0 ±4.7%b  | 4253 ±181b |
| NC125              | 26.0 ±2.4%ab  | 28.0 ±0.7%b  | 4513 ±129b |
| NC150              | 28.0 ±2.9%ab  | 32.0 ±2.7%ab | 5620 ±94a  |
| NC175              | 31.0 ±0.9%a   | 38.0 ±1.1%a  | 5929 ±52a  |

Note: Diets: PC - positive control, 15% fish meal (FM), 10% cottonseed meal (CSM); NC - negative control, 11% FM, 14% CSM; NC125 - NC + 125 mg/kg protease; NC150 - NC +150 mg/kg protease; NC175 - NC +175 mg/kg protease;

Figure 1: Principal component analysis of the variables and their correlation with the level of dietary protease



# **DRY MATTER AND PROTEIN APPARENT DIGESTIBILITY COEFFICIENTS OF DIETS CONTAINING MARINE BY-PRODUCT MEALS FOR JUVENILE LONGFIN YELLOWTAIL *Seriola rivoliana*, AT TWO WATER TEMPERATURES AND USING THREE FECES COLLECTION METHODS**

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Marine by-products such as Penshell (*Atrina maura*) and Catarina scallop (*Argopecten ventricosus*) viscera, and shrimp heads (*Litopenaeus stylirostris*) are rich in high quality protein and other nutrients. Dietary inclusion of Penshell viscera or shrimp head meals to partially replace fishmeal in diets for *Seriola rivoliana*, improves growth. However, little is known about the bioavailability of nutrients in diets containing by-products meals for this species. Several studies have demonstrated that protein apparent digestibility coefficients are affected, among other factors, by water temperature and feces collection method.

Here, a feeding trial with juvenile longfin yellowtail ( $495.1 \pm 9.4$  g) was conducted to determine dry matter and protein apparent digestibility coefficients under different culture conditions and feces sampling methods. Five diets containing chromic oxide as inert marker were tested: a Reference diet (50% CP, 13% L) containing fish meal as main protein source, three diets containing 12.5% of the experimental by-product meals, and one diet (SCP) containing 12.5% of each of the experimental meals. The juveniles were produced at CIBNOR, La Paz. Each dietary treatment consisted of three replicates (600-L tanks) containing 6 fish per tank. Fish were fed to apparent satiation 3 times per day. The juveniles were reared at two temperatures (25°C and 21°C) and feces were collected by three methods: siphoning, stripping, and dissection.

Preliminary results of protein apparent digestibility coefficients are shown in Table 1.

**Table 1.** Protein apparent digestibility coefficients (%) in *Seriola rivoliana* fed diets containing by-product meals at two temperatures and by three feces collection methods.

| Feces collection method | Diets     |             |                  |          |          |
|-------------------------|-----------|-------------|------------------|----------|----------|
|                         | Reference | Shrimp head | Catarina scallop | Penshell | SCP      |
| Siphoning 25 °C         | 84.4±1.6  | 89.0±1.1    | 87.3±1.0         | 92.3±0.2 | 90.0±0.3 |
| Siphoning 21°C          | 82.5±5.0  | 91.3±0.1    | 87.5±1.7         | 93.2±0.5 | 89.4±0.6 |
| Stripping 25 °C         | 84.9±1.0  | 85.6±0.4    | 88.6±0.9         | 88.9±0.1 | 85.5±1.1 |
| Stripping 21 °C         | 88.8±0.5  | 88.3±2.2    | 88.3±0.9         | 85.7±1.4 | 86.4±1.5 |
| Dissection 21 °C        | 67.8±0.9  | 77.9±1.9    | 78.1±4.0         | 79.0±2.1 | 72.8±2.9 |

## CREATING BETTER EDUCATIONAL VIDEOS FOR EXTENSION AND OUTREACH

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Welcome to the video age. The spread of high speed internet access and simple video editing software makes the creation and delivery of online educational videos easier than ever. YouTube videos provide an excellent mechanism for Extension specialists to share information and reach a large potential audience. Putting a Power Point presentation in video format does not make the most of this medium. The creation of good video content requires careful planning and practice. The average attention span of most adults is 15 minutes and students approximately 7-10 minutes. Most lecture presentations, however, last 30 minutes to an hour. How can you get your point across as quickly as possible? We must learn to provide entertainment in addition to education. YouTube suggests that the first 15 seconds of a video are critical for engagement and retention of the audience.

A central figure or “hero” and a story with a beginning, middle and end will go a long way towards retaining the attention of the audience. Join us for a discussion these and other simple tips and techniques that can improve your ability to create watchable or perhaps even enjoyable educational Extension videos.

## **NAVIGATING THE DEMANDS OF RESEARCH AND INDUSTRY NEEDS: TOOLS FOR STUDENTS AND EARLY CAREER RESEARCHERS**

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Moving into the business environment of aquaculture companies, and engaging with industry representatives, can often be a challenge for recent graduates and early career researchers. Undergraduate education and research higher degree training are critical to provide graduates with the skills required to tackle the challenges faced by the aquaculture industry. However, embarking on the journey to establish a research career and to operate effectively to meet industry needs requires more from graduates than the experiences obtained in a university setting. Many tools are available to navigate the apparently conflicting demands of quality research and industry needs.

Case studies of industry engagement will be used to demonstrate various tools for early career aquaculture researchers. These tools include: understanding industry drivers (why do scientists and industry seem to speak different languages?); situation analysis (what are the current operating processes and environment?); identification of critical points (where are the issues and how important are they?); establishment of trust (how to, and how not to, communicate?); working with a network of collaborators (does an issue need a broader set of skills or more people to find a solution?); prioritising research (what to do first and why?); delivering research with industry (what to think about when managing expectations?); communicating research findings (how to share the significance of research?); delivering solutions (how to work with industry to make positive change?).

The presentation is designed to equip students and early career researchers with practical tools to apply in their professional lives that enable the delivery of relevant research to solve key industry needs.



## HATCHERY PRODUCTION OF *Tripneustes gratilla* FOR SEAWEED BIOCONTROL AND CORAL REEF CONSERVATION

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The sea urchin *Tripneustes gratilla* is raised as a biocontrol agent to mitigate the effects of invasive seaweeds in Kaneohe Bay, Hawaii.

In the 1970s the eucheumoid seaweeds *Kappaphycus alvarezii*, *Kappaphycus striatum* and *Eucheuma denticulatum* were introduced to Hawaiian waters during aquaculture research trials. These seaweeds have become invasive pest species smothering native corals and contributing to the decline of coral reef habitat within Kaneohe Bay. Depressed populations of grazing animals have resulted in low herbivory allowing the eucheumoids to grow relatively unchecked within the Bay. The native collector urchin *Tripneustes gratilla* was identified, tested and found to be an effective biocontrol species to reduce the spread of invasive seaweeds.

*Tripneustes gratilla* is raised at the Anuenue Fisheries Research Center in Honolulu. Wild broodstock are collected and spawned monthly. First feed is administered three days after fertilization. Larvae are grown in 200L cone-bottom tanks with mild aeration. Larvae are maintained in filtered, UV treated seawater within a temperature range of 25C to 26C. Water exchanges or tank changes are performed daily. Larvae are fed on a mixed diet of *Rhodomonas sp.* and *Chaetoceros muelleri*. Competency in a majority of larvae is usually observed at 23 days post-fertilization. Competent larvae are moved to settlement tanks for metamorphosis and grow-out. Settlement tanks are prepared with natural biofilms. Post-larval urchins graze on biofilms for eight to twelve weeks. At 5mm to 7mm juvenile urchins are switched to a diet of cultured macroalgae. Within three to five weeks of feeding on macroalgae, urchins more than double in size and are ready for outplanting in Kaneohe Bay.

When urchins are three to five months of age and at a size of 15mm to 20mm, they are released onto coral reefs. Previously invaded reefs remain >95% clear of nuisance seaweed once urchins are present.

## CHANGES IN THE FECAL MICROBIOTA OF THE WHITE SHRIMP *Litopenaeus vannamei* GENERATED BY PRESENCE OR ABSENCE OF *Ulva ohnoi* IN DIET

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Seaweeds are one of the natural resources with more active compounds, which can be used to develop functional foods that improve the health and production variables. The aim of this study was to demonstrate that the change from a diet with 4% of seaweed *Ulva ohnoi* meal to a control without *Ulva* modifies the fecal microbiota of *Litopenaeus vannamei*. A metagenomic analysis amplifying the 16s rRNA V3 region of fecal samples from 4 shrimps confined individually and fed successively for four days with two treatments (*Ulva* diet and control) was carried out to track the change in fecal microbiota of each shrimp. DNA extractions were done with CTAB buffer. Sequencing was carried out on Ion Torrent PGM (Applied bio systems) equipment. Characterization and analysis of the bacterial community structure were carried out using owned scripts and the software QIIME 1.9, and the statistics with the STAMP software. Operational taxonomic units (OTU) were classified at a similarity level of 97%. The fecal bacterial community for both treatments was dominated by nine families: Pseudoalteromonadaceae, Vibrionaceae, Alteromonadaceae, Alcaligenaceae, Pasteurellaceae, Rhodobacteraceae, Colwelliaceae, Flavobacteriaceae, y Campylobacteraceae. When comparing the sequences obtained from feces collected under the two different treatments in same shrimp (individual comparison), proportions of families and genera were significantly different ( $p < 0.05$ ), the Pseudoalteromonadaceae appearing in greater proportion with *Ulva*, while the Vibrionaceae, Alteromonadaceae and Pasteurellaceae were dominating under the control diet. The genera *Vibrio* (most frequent pathogen bacteria in shrimp culture) and *Pseudoalteromonas* appeared in less proportion under the *Ulva* treatment. When comparing the samples in groups according to the treatment, only the Vibrionaceae family and *Vibrio* genus were significantly less abundant under the *Ulva* treatment. This study is the first to demonstrate, through metagenomics, the effect of green seaweed *Ulva ohnoi* as functional ingredient able to modify the fecal microbiota with high potential to improve the nutrition and health of cultivated shrimp.

## EFFECT OF DIET SUPPLEMENTATION WITH GREEN SEAWEED ON GROWTH, NITROGEN RETENTION AND IMMUNE RESPONSE OF THE SHRIMP *Litopenaeus vannamei*

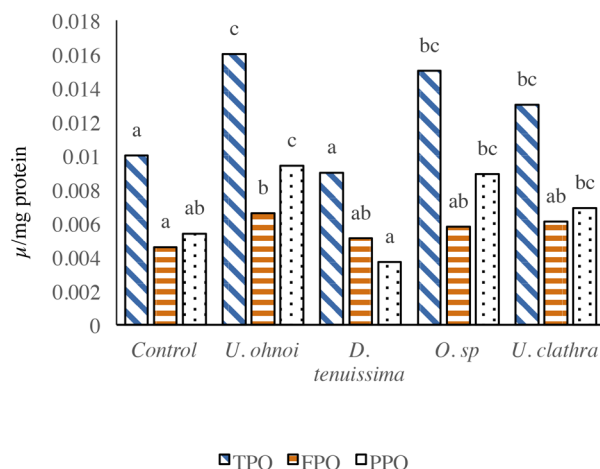
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Several studies have shown that feeding diets supplemented with green seaweeds (such as *U. clathrata*, *U. lactuca*, *U. reticulata*, and *U. prolifera*) to shrimp offer an effective protection against some pathogenic bacteria and virus. There are other species of green macroalgae that have not been evaluated in shrimp and may promote growth or immune response. This study investigates whether *Ulva ohnoi*, *Oedogonium sp* and *Derbessia tenuissima* green seaweeds included at 4% in diets for white shrimp *L. vannamei* juveniles may improve growth, nitrogen retention and immune system as efficiently as *Ulva clathrata*.

A 45 days feeding trial was conducted to evaluate a control diet formulated to contain 40% crude protein and 9% crude lipids, and four more diets manufactured by supplementing the control formula with 4% dehydrated *U. ohnoi*, *D. tenuissima*, *Oedogonium sp* (provided by MBD Australia) or *U. clathrata* (provided by Aonori Aquafarm Mexico.). Shrimp juveniles averaging  $70 \pm 1$  mg were randomly allotted to 25 fiberglass tanks (five treatments in quintuple groups) and were fed the test diets to apparent satiation three times a day.

Shrimp fed diets containing green seaweed showed similar growth, feed intake, biomass, and survival than shrimp fed control diet. The inclusion of *U. clathrata* improved feed conversion ratio (1.55 versus 1.70-1.85). NRE was significantly higher in shrimp fed diets containing *U. clathrata* seaweed meal (31.1% versus 27.4-27.3%). Survival was 100% with all the treatments. Shrimp fed *U. ohnoi*, *Oedogonium sp*. and *U. clathrata* diets exhibited higher total phenoloxidase and prophenol oxidase activities than shrimp fed *D. tenuissima* and control diets. Free phenol oxidase activity was slightly increased by the inclusion of all seaweed meals tested. Dietary seaweed meal did not modify total haemocyte count, nor hyaline, semi-granular and granular cells proportions. The tested green seaweeds, specially *Ulva* species, are recommended as immune stimulant for shrimp diets.



## USES AND ADVANTAGES OF MULTITROPHIC EARTH POND AQUACULTURE IN SOUTH EUROPEAN COUNTRIES: RESEARCH FROM PORTUGAL

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Abandoned salt marsh areas still exist in Portugal and other South European countries. Salt-production, extensive and semi-intensive aquaculture, where the main activities in those areas. Restrictions for improving production and strong regulations to mitigate environmental impacts maintain this scenario. Nevertheless recovery of these areas has been discussed in several past European projects (SEACASE, SEAFARE). One strategy that have emerged from these projects and contributes to increase the value of such areas is Integrated Multi-Trophic Aquaculture (IMTA), combining several species at different trophic levels. At IPMA's Aquaculture Research Station in Olhão have been performed since 2010 to find the adequate combinations between marine fish species, oysters and since 2015 *Ulva* species.

Since the concept if IMTA is flexible and can be adapted to different conditions and objectives, with sub-systems separated (Fish tanks + Seaweeds tanks + bivalves tanks) or together like at EPPO, we think that this strategy can be implemented as an ecosystem service and also a compensatory measure for the implementation of intensive systems in salt marsh areas. Finding the key species and its trophic interactions under these coastal earth ponds will be essential to evaluate IMTA ability to serve as an efficient tool in Coastal Marine Management.

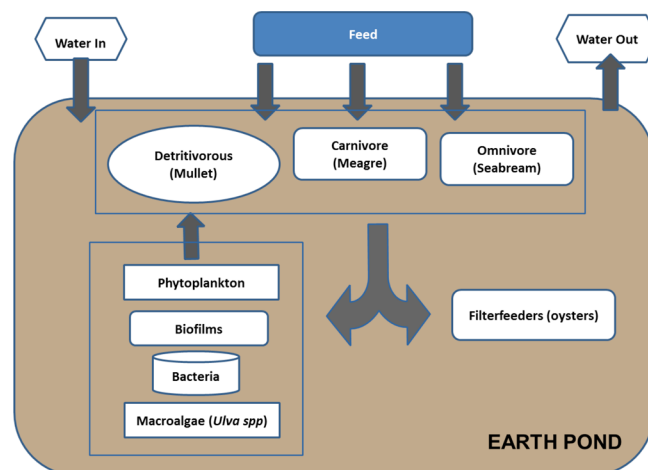


Figure 1 – Diagram of IMTA combinations at EPPO earth ponds.

Acknowledgements: This work has been funded by the COFASP European project IMTA-EFFECT (COFASP- 976) and DIVERSIAQUA project (MAR2020, Portugal) .

## DIETARY INCLUSIONS OF ACTI-MEAL IMPROVES THE GROWTH AND FEED UTILISATION OF GREENLIP ABALONE *Haliotis laevis*

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There is considerable scope in the abalone industry to utilise waste products, such as Acti-Meal, in abalone diets. Acti-Meal is a by-product of the wine industry, and is a dried, milled, steamed distilled grape marc meal product produced by Tarac Technologies, SA, Australia. Notably this product is considerably cheaper than currently used land based macro ingredients, and is available in commercial quantities.

In this 90 day study, the growth and feed utilisation of greenlip abalone (1.82 g) fed diets containing graded levels of Acti-Meal (0, 5, 10, 15 and 20%) were examined. Diets were formulated to contain 35% crude protein, 5% crude lipid and 17.5 MJ kg<sup>-1</sup> gross energy. A commercial diet was also fed to abalone, and this was compared to the 0% diet. Growth and feed utilisation of abalone fed the commercial diet and the 0% diet were similar. There was a significant positive effect of Acti-Meal inclusion on the growth performance and feed utilisation of greenlip abalone. Abalone fed any inclusion level (5, 10, 15 and 20%) exhibited superior growth to abalone fed the 0% diet. In addition abalone fed diets with Acti-Meal had superior FCRs compared to the 0% diet.

Acti-Meal is a commercially available, viable, option for inclusions into formulated abalone diets. Not only did the addition of Acti-Meal consistently improve growth performance and feed utilisation, but the product is also considerably cheaper than currently used land based ingredients. We recommend dietary inclusions of 5-20% Acti-Meal to improve abalone growth and feed utilisation. As this ingredient is currently commercially available we also recommend a pilot scale on-farm growth trial over an extended period be conducted, prior to feeding diets containing this product on a commercial basis.

## SETTLEMENT, METAMORPHOSIS AND POST-SETTLEMENT SURVIVAL OF THE SEA URCHIN *Tripneustes gratilla* FOR AQUACULTURE PRODUCTION

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Successful larval settlement and metamorphosis has in many instances been linked to associations with the recognition of specific substrates or substratum-specific biochemical signals. In many intensive aquaculture systems, the required morphogenetic inducing substances are often absent. As a consequence, these aquaculture operations suffer from costly and extensive early post-larval mortality, which can be linked to abnormal development during metamorphosis. This study examined the effects of a range of inductive substrates and chemicals on larval settlement and metamorphosis as well as post-settlement survival and growth of the sea urchin *Tripneustes gratilla*. The effect of each settlement substrate or inducer was evaluated by quantifying the number of competent larvae that successfully completed metamorphosis within a 48h period. We tested a range of benthic microalgal species (*Amphora* sp.; *Cocconeis* sp.; *Navicula* sp.; *Nitzschia* sp.; Tank diatom communities (TD)), macroalgal species (*Ulva* sp., *Ulvellla* sp.), bacterial bio-films and chemicals (histamine, dibromoethane,  $\gamma$ -aminobutyric acid (GABA), *Ulva* extracts). We demonstrated that a higher percentage of larvae successfully completed metamorphosis in association with a natural diatom community (98 $\pm$ 2.0%) and fresh seaweed *Ulva* (68 $\pm$ 10.7%), compared with only 20% of larvae successfully completing metamorphosis in association with either of the benthic microalgae species tested (Fig 1). Settlement substrates also had an effect on post-settlement growth of urchins, with the macroalgal crust, *Ulvellla lens*, producing the best growth over a 30 day period. These findings will contribute towards more consistent and successful production of *T. gratilla* juveniles and the development of echinoculture in South Africa.

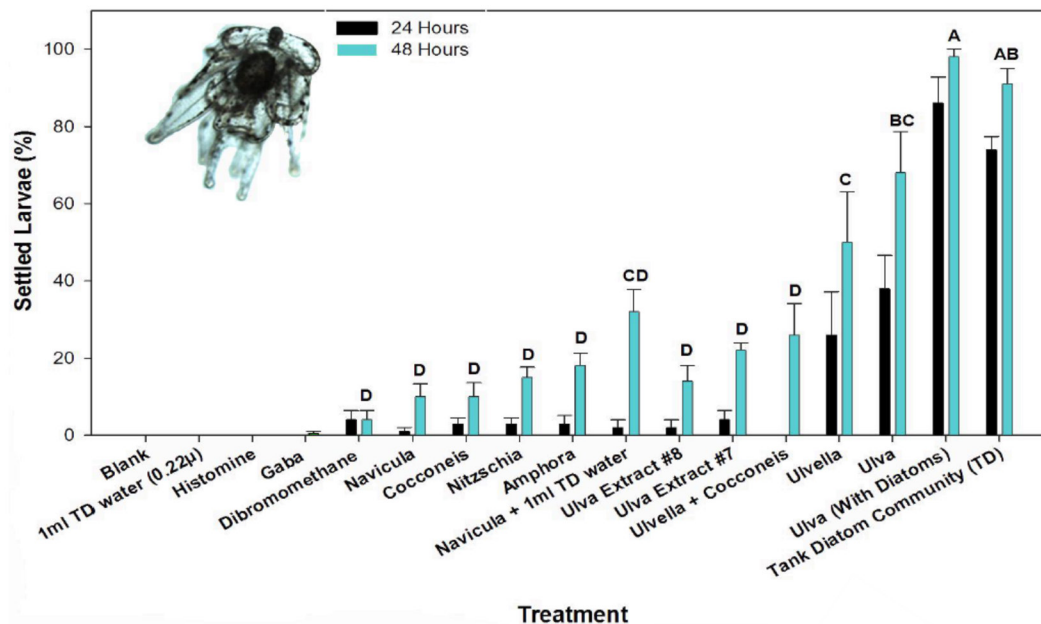


Figure 1. Mean ( $\pm$ SE) number of larvae that settled at 24 & 48h in presence of different settlement cues. Urchins were considered settled only if full metamorphosis had occurred and not just larval attachment.  
(Blank = filtered seawater; TD = Tank Diatom Community; Gaba =  $\gamma$ -Aminobutyric acid)

## AN OVERVIEW OF ECHINOCULTURE DEVELOPMENT IN SOUTH AFRICA

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*Tripneustes gratilla* has been proposed as a viable candidate for aquaculture in South Africa due to its fast growth rate, early maturation and high gonadal production. Commercially valuable urchin gonads should be large in size, contain few to no gametes, have a firm texture, and should be bright yellow or orange in colour. High quality urchin gonads can have a whole sale value of up to \$600 per kg. Key areas that are, however, hampering the successful commercial scale culture of *Tripneustes* in South Africa, and abroad, include: (1) production of cost-effective diets; (2) development of protocols for uniform conditioning of adults prior to harvest; (3) improving larval survival, settlement and post-settlement survival. Our research has focused on addressing each of these areas. We have developed artificial feeds supplemented with macroalgae and have optimized feeding regimes to produce high quality gonads. A formulated feed supplemented with 20% dried *Ulva* (20U diet) was shown to produce high quality gonads in terms of both size and colour. Moreover, dietary *Ulva* supplementation significantly increased protein digestibility, the chemosensory properties of a formulated feed and feed consumption rates. Feeding regimes have also been developed and optimized for full-life cycle grow-out of urchins. We showed that somatic growth was similar for urchins fed fresh *Ulva* (FU) or a formulated feed (20U), however urchins fed FU had significantly smaller gonads and required an additional 6 weeks of feeding with formulated feed (20U diet) to attain gonads of a marketable size; similar to the gonads of urchins continually fed the 20U diet. Significant progress has been made with regards to improving larval growth and survival, settlement success and post-settlement survival of larvae, and our group can now consistently produce large numbers of high quality larvae and juveniles. Key findings of this research will be presented and the implications for the development of a cost-effective and successful echinoculture industry in South Africa will be discussed.



## PROTEC FOR TILAPIA – FISH HEALTH MODULATION THROUGH NUTRITION

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The African aquaculture industry is developing hastily, and its major economic losses have, to date, been primarily due to disease, which remains a key constraint to its continued growth. As the industry's rate of expansion intensifies, the benefits of high quality feeds that could offer enhanced fish growth and improved disease resistance, become more apparent.

Hence, substantial progress has been made during the past decades towards understanding the relationships between dietary formulation, growth efficiency, stress, the immune system, and prevention / control of disease. It is widely recognised that nutritional modulation can have a profound effect on the overall performance of fish, and that certain feed additives, besides satisfying the dietary nutrient requirements for maximum growth, can provide increased immunocompetence and in turn increase resistance to disease.

Feed additives may therefore be an effective means for reducing the levels of mortality in aquaculture due to disease.

To minimise the impact of overwhelming disease outbreaks, Skretting ARC has developed PROTEC™ for tilapia. This support-diet has shown to optimise growth, improve nutrient utilisation, decrease production costs, and help maintain “normal health”. The effects and potential role of the main components from this functional diet on the overall health performance of tilapia constitute a major topic of this presentation. The effects of such diet per experimental challenge with *Streptococcus agalactiae* are also shown.

## ISOLATION OF *Nucleospora braziliensis* FROM NATURAL INFECTION OF NILE TILAPIA (*Oreochromis niloticus*) IN CELL CULTURE

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Microsporidia are found in the environment in worldwide and some species can infect animals, such as fish and shrimp, as also humans. Among fish diseases caused by microsporidium, *Nucleospora* genus is very important because it can cause serious lesions and highly mortality levels. During November 2014 and March 2015, it was detected a new species of *Nucleospora* infecting *Oreochromis niloticus*, which was named *N. braziliensis*. For other studies such as genome, molecular characterization, and pathogeny, this study aimed isolate this new microsporidium in VERO cell line.

For isolation, it was inoculated 1 g of kidney of *O. niloticus* naturally infected with *N. braziliensis* in VERO cell line established in the laboratory. The confirmation of infection was performed with polymerase chain reaction with the primers ES-1a, ES-2a, ES-3a, and ES-4a, as described by Barlough *et al.* (1995). The PCR products were electrophoresed on a 1.5% agarose gel stained with 1% SYBR safe (Thermo Fisher Scientific®), alongside a High Ranger 100-bp DNA ladder (Norgen Biotek Corp.®). The 407-bp amplicons produced with the primers ES-3a and ES-4a were excised from the gel and purified with an Illustra Microspin™ S-400 HR Columns Kit (GE Healthcare®) according to the manufacturer's instructions.

After purification of the amplified products, Sanger sequencing was performed. For this purpose, the purified amplicon was sequenced in both directions using Big Dye™ Terminator Cycle Sequencing Kit (Applied Biosystems) on an Applied Biosystems capillary 3500 Genetic Analyzer. The quality of the electropherograms was assessed in Sequencing Analysis version 5.4 (Applied Biosystems).

In the day 4 after inoculation of tissue infected in VERO cells line was detected a product of 407 bp, compatible with *Nucleospora* spp. After sequencing, it was identified that the isolation worked well, since we found 97% of identity with *Nucleospora salmonis* (genbank: AF185998) and 100% with *N. braziliensis*. These isolated microsporidians will be used for molecular and pathogeny mechanisms studies in the laboratory.

### Reference:

Barlough JE, McDowell TS, Milani A, Bigornia L, Slemenda SB, Pieniazek NJ, Hedrick RP. Nested polymerase chain reaction for detection of *Enterocytozoon salmonis* genomic DNA in Chinook salmon *Oncorhynchus tshawytscha*. Diseases of Aquatic Organisms. 23(1):17-23, 1995.

# **MONOGENEAN INFESTATION IN *Oreochromis niloticus* CULTIVATED IN NET CAGES IN DIFFERENT RESERVOIRS IN SÃO PAULO STATE, BRAZIL**

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Tilapia (*Oreochromis niloticus*) farming has a great economic importance to Brazilian fish industry. However, diseases affecting cultured fishes are a serious problem leading to economic losses. Gill monogenean infestations is the main health problem in tilapia fish farms in Brazil. The aim of this study is to evaluate the occurrence of monogeneans in tilapias of six farms from three different reservoirs (Parapanema, Tietê, and Paraná/Grande rivers) in São Paulo State, Brazil, in spring and autumn, when the mortality rate is usually high. Parapanema and Grande/ rivers are considered oligotrophic and Tietê is mesotrophic river.

Monogeneans were surveyed in the gills of 120 fish (60 in each season). All monogenean species recovered were characterized by morphological and molecular methods. The prevalence of parasites was similar in all fish farms and seasons. However, different parasite abundances and intensities of infestation considering the seasons and fish farms were observed. Monogeneans were more abundant in the mesotrophic river during spring, while in the oligotrophic rivers in autumn. The monogeneans species recovered were *Cichlidogyrus sclerosus*, *C. halli*, *C. thurstone*, *Cichlidogyrus* sp. and *Scutogirus longicornis*. Prevalence, abundance and intensity of infection for each monogenean species varied according to fish farms and seasons, but a clear pattern of infestation was not detected. *Cichlidogyrus sclerosus* and *C. halli* were the most abundant species in all fish farms. Abiotic parameters of the water or technical management of fish farms could contribute with the establishment of infestation rate.

**Table 1.** Parasitological analyses (prevalence [P], mean intensity of infestation [MII], and mean abundance [MA]) of monogenean infestation in *Oreochromis niloticus* from six fish farms (FF) in Brazil during spring and autumn. N - number of monogeneans, SE - standard error, R - range (minimum and maximum values).

| FF | Spring (n = 60) |     |                                     |                                     | Autumn (n = 60) |     |                                    |                                    |
|----|-----------------|-----|-------------------------------------|-------------------------------------|-----------------|-----|------------------------------------|------------------------------------|
|    | N               | P   | MII ± SE (R)                        | AM ± SE (R)                         | N               | P   | MII ± SE (R)                       | AM ± SE (R)                        |
| 1  | 1631            | 92  | 30.2 ± 4.2 <sup>a</sup><br>(2-118)  | 27.2 ± 4.0 <sup>a</sup><br>(0-149)  | 3708            | 98  | 62.8 ± 7.0 <sup>b</sup><br>(0-202) | 61.8 ± 7.0 <sup>b</sup><br>(0-202) |
| 2  | 2580            | 100 | 43.0 ± 3.4 <sup>a</sup><br>(2-118)  | 43.0 ± 3.4 <sup>a</sup><br>(2-118)  | 1268            | 100 | 21.1 ± 2.3 <sup>b</sup><br>(1-83)  | 21.1 ± 2.3 <sup>b</sup><br>(1-83)  |
| 3  | 5512            | 100 | 91.8 ± 8.7 <sup>a</sup><br>(10-356) | 91.8 ± 8.7 <sup>a</sup><br>(10-356) | 2297            | 98  | 38.9 ± 3.8 <sup>b</sup><br>(0-113) | 38.3 ± 3.8 <sup>b</sup><br>(0-113) |
| 4  | 5132            | 100 | 85.5 ± 8.7 <sup>a</sup><br>(11-283) | 85.5 ± 8.7 <sup>a</sup><br>(11-283) | 2354            | 100 | 39.2 ± 4.1 <sup>b</sup><br>(4-137) | 39.2 ± 4.1 <sup>b</sup><br>(4-137) |
| 5  | 1708            | 100 | 29.7 ± 2.8 <sup>a</sup><br>(2-122)  | 29.7 ± 2.8 <sup>a</sup><br>(2-122)  | 3480            | 100 | 58.0 ± 8.1 <sup>b</sup><br>(7-372) | 58.0 ± 8.1 <sup>b</sup><br>(7-372) |
| 6  | 5272            | 100 | 87.8 ± 10.1<br>(5-465)              | 87.8 ± 10.1<br>(5-465)              | 4529            | 100 | 75.4 ± 7.5<br>(10-238)             | 75.4 ± 7.5<br>(10-238)             |

**Comparisons of fish farms:** Spring - MII: 3 = 4 = 6 > 1, 2 e 5, e 1 > 2, H = 102,783, p ≤ 0,001; MA: 3 = 4 = 6 > 1, 2 e 5, e 1 > 2, H = 111,444, p ≤ 0,001; Autumn - MII: 1 < 2 < 3, 4, 5 e 6, e 6 > 3 e 4, H = 56,724, p ≤ 0,001; MA: 1 < 2 < 3, 4, 5 e 6, e 6 > 3 e 4, H = 55,773, p ≤ 0,001.

## MONITORING CYANOBACTERIA IN LARGE FRESHWATER RESERVOIRS WITH OPEN CAGE AQUACULTURE SYSTEMS BY *IN SITU* FLUORIMETRY

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Good water quality is decisive for cage aquaculture, and algal blooms involves risks of spoiling production by cyanobacterial metabolites that can result on off flavors and toxin accumulation. Blooms are not easily predictable and highly variable in space and time, making timely detection of cyanobacterial bloom formation a relevant topic. New technologies based on fluorescence probes are starting to be employed for *in vivo*, real time assessments of cyanobacterial populations, opening the possibility for early detection of algal blooms, allowing better management of fish production. The aim of this study was to assess the applicability of a submersible *in situ* fluorescence meter for the measurement of cyanobacterial population in cage aquaculture farms installed in large freshwater reservoirs in Southeastern Brazil.

Fluorimetry results of phycocyanin and chlorophyll levels were compared to standard microscopical counts of phytoplankton and cyanobacteria. Samples were collected in 12 sites, at six open cage, grow out systems of Nile tilapia. The studied farms are sited in three large, tropical hydroelectrical reservoirs. Simultaneously, vertical profiles were sampled using a sonde YSI EXO2 equipped with a fluorescence meter for chlorophyll and phycocyanins (BGA-PC) and also sensors for measuring temperature, conductivity, pH, turbidity, dissolved oxygen. Total cyanotoxins were determined by HPLC equipped with a UV-vis detector.

Regression analysis showed that cyanobacteria density is significantly related to *in situ* fluorescence readings (BGA-PC) (Figure 1). Accurate detection of cyanobacteria at the first alert level proposed by the World Health Organization (WHO), which is 2000 cells/mL, is considered the minimum acceptable detection limit for submersible fluorescence probes. Water turbidity did not biased *in vivo* measurements, but higher chlorophyll levels were related to underestimation of phycocyanins. Toxins levels are also significantly correlated to phycocyanin and chlorophyll levels. Our results indicate that submersible fluorescence probes are useful for real time monitoring systems for aquaculture areas, able to provide early warning that could trigger further actions such as additional analysis of phytoplankton to confirm bloom occurrence, and analysis of toxins and off flavors to assure the quality of fish production.

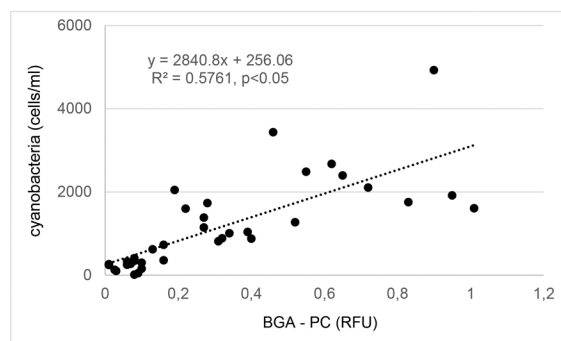


Figure 1. Comparison of probe raw readings of cyanobacteria *in situ* (RFU, relative fluorescence unit) and microscopic counts of cyanobacteria (n=36).

## METABOLOMICS IS A CRUCIAL TOOL FOR AQUACULTURE: AN ABALONE EXAMPLE

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Selection to domesticate aquatic animals such as fish and abalone for optimal meat production needs to be related to the farm conditions to maximise growth and survival, and these conditions are still evolving. While many dietary components have been optimised, farms are trialling oxygen supply, tank design changes and other factors. Oxygen is critical in intensive aquaculture systems, because dissolved oxygen is easy to deplete with a high density of animals and the supply decreases as temperatures rise. Oxidative metabolism is the engine for growth: without it, animals will lose weight as they break down proteins etc. for energy. Yet in many animals we don't know how oxidative metabolism varies with temperature, oxygen supply and also diet, particularly antioxidant supplements. The control of oxidative energy production is sensitive to temperature, and at higher temperatures it may become very inefficient, slowing growth.

Metabolomics research allows us to identify how food is digested into components and these are used to build animal tissue. We can then study alterations due to temperature, oxygen, antioxidant supply, etc. The wave of this research is starting to build in abalone aquaculture. In only the second published study of abalone metabolomics, we have used <sup>1</sup>H-NMR metabolite profiling of abalone food, faeces and digestive glands after short-term starvation as a pilot to examine the digestive process in the hybrid abalone used in Australian aquaculture.

Multivariate analysis revealed the starved abalone had a different metabolic profile than those that were continuously fed after 28 days and 56 days. A suite of key oxidative metabolites were reduced in the starved group. But abalone appeared to adjust their metabolism to starvation between 28 and 56 days, as there were fewer differences after 56 days. The rate of oxidative metabolism may decline as food declines, although there was no sign of a switch away from oxidative energy production. Glucose continued to decline from 26 to 56 days of starvation, and an important osmolite, N,N-dimethylglycine, increased.

Abalone use glycogen as an energy store, but after 56 days glycogen levels had declined to similar levels in the digestive glands of both starved and fed abalone, although initially they declined faster in the starved group. This suggests regulation of the levels in this gland, possibly using the foot as the glycogen store. The faeces produced during initial starvation still contained many of the amino acids present in the artificial feed, and the most depleted metabolites in faeces suggest further refinements of the diet are possible.

Further work is needed to show how increased temperatures affect the metabolic process in these abalone, and whether there is an increased reliance on breaking down reserves for energy. The role of dissolved oxygen concentrations also needs investigation. Metabolomics has already optimised human athletes, and is poised to revolutionise the way we manage aquaculture.

## MICRO-ORGANISMS ASSOCIATED WITH *Ulva* CULTIVATED IN AN INTEGRATED AQUACULTURE SYSTEM WITH ABALONE, AND IMPLICATIONS FOR BIOSECURITY

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In integrated aquaculture of abalone, *Haliotis midae*, and *Ulva* spp. in South Africa abalone effluent water serves as a nutrient source for the cultivation of *Ulva*, and the *Ulva* produced from these systems serves as feed for the abalone. This partial recycling however raises several biosecurity concerns. Due to the importance and potential benefits of algae within integrated aquaculture systems, it is becoming increasingly important to disentangle the relationship between algae, fungi and bacteria.

This study reports on the biosecurity implications associated with the use of *Ulva* as a biofilter and feed within these systems by assessing the bacterial and fungal communities associated with *Ulva* and its environment. Water and *Ulva* samples were collected from an integrated abalone farm and assessed via a culture and a non-culture based approach. Samples were collected from both a fertilised seawater system and an effluent system. The water samples were collected at the inlets and outlets of each system. The culture based approach utilised three selective media for the isolation and quantification of culturable bacteria, namely Tryptic Soy Agar (TSA), thiosulfate-citrate-bile-sucrose (TCBS) agar (vibrio selective), and Ulvan agar plates where the main carbohydrate of *Ulva* was utilized as the main carbohydrate source. Post isolation, bacteria and fungi underwent 16S and 18S rDNA gene analysis, respectively, for identification. The non-culture based approach used Illumina next generation sequencing, which enabled the assessment of the entire bacterial community within water and *Ulva* samples.

*Ulva* has the potential to significantly reduce the bacterial load of abalone effluent water systems, with a decrease in CFU/mL<sup>-1</sup> recorded on the TSA and TCBS in all effluent systems (Fig. 1). The composition and relevance of the identified bacteria and fungi as well as the alpha and beta diversity of the 16S and 18S rDNA gene libraries will be discussed.

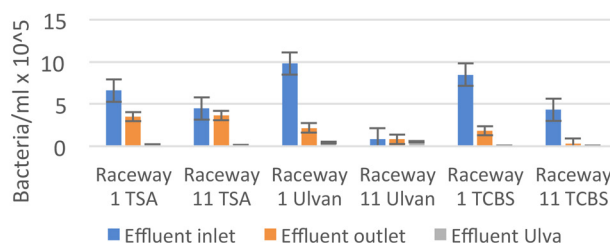


Figure 1. Effluent water and Ulva bacterial loads

## **THE OINTMENT IN THE FLY: PROSPECTS FOR USE OF BLACK SOLDIER FLY (*Hermetia illucens*) IN AQUAFEEDS**

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Various studies on Black Soldier Fly meal and oil have proven it as rich sources of nutrients with favourable amino acid and fatty profiles that can contribute to more sustainable feeds for aquaculture. Beyond its nutritional value, it also offers functional value through chitin-derived prebiotics and antimicrobial triglycerides and peptides. The aim of this presentation is to provide a summary of research and potential application of Black Soldier Fly meal in aquafeeds.

## **EVALUATION OF BLACK SOLDIER FLY OIL FOR USE IN DIETS OF AFRICAN CATFISH *Clarias gariepinus***

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The reliable and sustainable supply of fish oil for use in catfish feeds has become under tremendous pressure due to the increasing demand but limited supply thereof as catfish production expanded - therefore the need to find more sustainable alternatives. Black soldier fly (BSF) oil has been identified as an interesting candidate for inclusion in fish oil replacement blends due to its naturally high content of medium chain triglycerides, potentially offering the benefits of improved energy utilisation and antimicrobial activity.

A 90-day trial was designed to evaluate two BSF Oils on production performance of juvenile African catfish. Treatments consisted of a control diet formulated with pilchard oil replaced at various levels (0, 25, 50, 75 and 100%) with two BSF oils. Each of the seven treatments were replicated 6 times. Results will be presented.



## TOXICITY OF CHITOSAN NANOPARTICLES AND ITS ROLE IN IMMUNE DEFENSE AGAINST *Aeromonas hydrophila* IN ZEBRAFISH LARVAE

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In this study, chitosan nanoparticles (CNPs) were synthesized by ionic gelation method and its toxicity, immune responses and disease resistance capacity were investigated in zebrafish larvae. Average particle size and zeta potential of CNPs were 181.2 nm and + 37.2 mV, respectively.

To test the toxicity of CNPs, zebrafish embryo at 3 hour post fertilization (hpf) were exposed to different concentrations (0, 5, 10, 20 and 30  $\mu\text{g/mL}$ ) of medium molecular weight chitosan (MMW-C) and CNPs. Hatching and survival rates of the larvae at 96 hpf were determined. Both MMW-C and CNPs exposed larvae showed the concentration dependent decreased hatching and survival rates compared to unexposed larvae. Furthermore, in microscopic observations, both MMW-C and CNPs exposed ( $>10 \mu\text{g/mL}$ ) larvae showed malformations of the embryos including spinal curvet, pericardial edema, hyperaemia and even unhatched embryos. At 5  $\mu\text{g/mL}$ , the hatching rate was almost similar in both treatments, however, the survival rate was lower in MMW-C compared to CNPs exposure, suggesting that the toxicity effect of CNPs on hatched larvae was minimal at 5  $\mu\text{g/mL}$  compared to MMW-C.

Zebrafish embryo exposed to CNPs (5  $\mu\text{g/mL}$ ) showed significantly ( $p < 0.05$ ) higher survival rate (at 120 hpf stage) against pathogenic *Aeromonas hydrophila* challenge (at 72 hpf stage) compared to CNPs non-exposed larvae (control).

Moreover, quantitative real time PCR (qRT-PCR) results showed that CNPs exposed larvae (at 96 hpf stage) induced the expression of immune related (IL1 $\beta$ , TNF $\alpha$ , IL 12, IL 10, CXCLC1c, CC-CHEM, lyzosome-C and  $\beta$ -defensin) and stress (HSP70) response genes. In contrast basal level or down regulated expression of antioxidant genes (glutathione S transferase, catalase SOD peroxiredoxin 4, txndr1) were observed. These results suggest that although CNPs can have toxic effects to the zebrafish larvae at higher doses, CNPs exposure at 5  $\mu\text{g/mL}$  enhances the immune responses and develop the disease resistance against *A. hydrophila* which could be due to its strong immune modulatory properties.

## **HOW DOES BRAND AFFECT DEMAND FOR CATFISH? AN ANALYSIS OF STORE-BASED SCANNER DATA IN THE UNITED STATES**

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Recent studies on frozen seafood products (Bronnmann and Asche, 2016; Chen 2016; Asche et.al., 2015; Asche et.al., 2013; Ahmad and Anders, 2012; Roheim et al., 2007; Roheim et al., 2011; Larkin et al., 2012) using scanner data revealed that brand and fish species were the main factors affecting retail prices. Other factors which add distinct value to seafood products were package size, product and processed forms. The present study investigates the influence of brands on consumer demand and retail prices of catfish in the United States at a disaggregated product and market level. It has quantified price premium for different brands, catfish products and sizes, and measured brand loyalty of catfish consumers in different market locations. It has analyzed market prices, purchase volume of catfish products under promotional schemes and regular price situations, and market share of different brands. Finally, the study has quantified brand elasticity of demand for catfish. The study is based on data collected for the period 2009 to 2013 by the Nielsen Company under the Expanded All Outlets Combined (xAOC) ScanTrack data programs. Ten metro markets, namely, Atlanta, Boston, Chicago, Detroit, Los Angeles, New York, Philadelphia, Pittsburgh, San Francisco and Washington D.C. are studied.

## REPLACEMENT OF MENHADEN FISH MEAL BY THE TUNICATE *Styela plicata* IN THE DIET OF JUVENILE BLACK SEA BASS

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Tunicates are potential new candidate alternative protein sources for use in fish diets. Commonly known as sea squirts, tunicates are suspension feeding marine invertebrates. Some species of tunicates, such as *Styela plicata*, are worldwide invasive species. They foul many hard substrates in estuaries and often foul shellfish aquaculture equipment, which results in increased effort and cost for the aquaculturists to remove them. The present study was conducted as an evaluation of the use of tunicate meal in replacement of fishmeal in the diet of juvenile black sea bass *Centropristis striata*. The experimental system consisted of eighteen 75-L aquaria supported by a recirculating system in a controlled-environment laboratory. Tunicates collected from a local shellfish hatchery and marina were freeze dried, ground into meal, and analyzed for proximate composition. Menhaden fishmeal and tunicate meal protein concentrations were analyzed to be about 59.7% and 26% dry basis, respectively. A control fishmeal-based diet (45% crude protein, 13% crude lipid) was formulated to contain 30% (total diet) menhaden fishmeal. Six different isoproteinic and isolipidic diets were formulated to replace 0.0%, 8.3%, 16.7%, 25.0%, 33.3% and 41.6% of fishmeal protein (FMP) by tunicate meal protein (TMP) by substituting 0, 2.5, 5, 7.5, 10 and 12.5% (total diet) menhaden fishmeal with 5, 10, 15, 20 and 25% (total diet) tunicate meal, respectively. Fish (7.1 g mean initial wt.) were stocked at a density of 15 fish per tank ( $N = 3$  per treatment) and fed the test diets daily to satiation for 55 days. Temperature (23 °C), salinity (32), pH (7-8) and dissolved oxygen (7 mg/L) were held constant. Fish growth performance and feed utilization were monitored. Proximate analyses of diets and fish tissue were conducted.

At the termination of the study, black sea bass showed no significant differences in survival (86.7-95.6%), mean weight (18.5-22.9 g), feed intake (0.29-0.37 g/fish/d), feed conversion ratio (1.29-1.71), protein efficiency ratio (1.36-1.77), or whole body proximate composition (protein, fat, moisture and ash), with marginally significant ( $0.05 < P < 0.10$ ) differences in specific growth rate (%/day) and in body weight gain (%) between diets 1 (0% TMP) and 5 (33.3% TMP) (Figure 1). The results suggest that juvenile black sea bass accept and grow well on diets replacing at least 41.6% fishmeal protein with tunicate meal protein without adverse effects on survival, growth, or feed utilization. Maximum replacement level of fishmeal protein with tunicate meal protein requires further study.

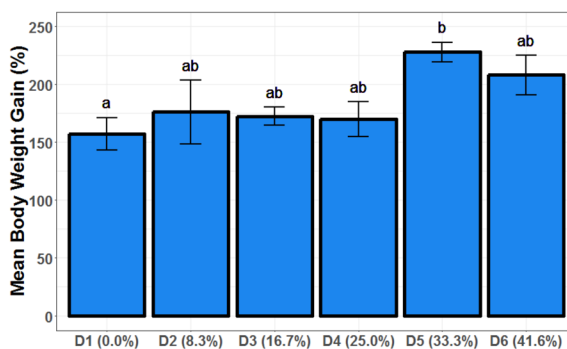


Figure 1. Final body weight gain (%). Diet number (% FMP replacement with TMP) is below each bar. Values are means ( $\pm$  SEM,  $N = 3$ ). Means not sharing a letter in common are marginally significant ( $0.05 < p < 0.10$ , Tukey HSD).

## BACULOVIRUS MEDIATED PRODUCTION OF INFECTIOUS TAURA SYNDROME VIRUS (TSV) OF SHRIMP IN NON-HOST CELLS

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Reverse genetics studies in RNA viruses can be greatly facilitated by the availability of an infectious cDNA clone. Genetic manipulation in Taura syndrome virus (TSV), a major viral pathogen of marine shrimp (*Penaeus sp.*), has been hampered due to the lack of a full-length genomic cDNA clone and the unavailability of an immortal cell line in shrimp. Here in, we show the construction of a recombinant baculovirus that expresses TSV in a non-host cells which is infectious to its natural host, shrimp. We cloned a full-length cDNA clone of the genomic RNA of TSV under the control of a shrimp viral promoter into a baculovirus expression system. Mature TSV as well as recombinant baculovirus were purified from baculovirus-infected Sf9 cells. Both the purified TSV and the recombinant baculovirus were infectious when injected into shrimp (*P. vannamei*). The clinical signs and histopathology in virus-injected shrimp manifested by the recombinant TSV and the baculovirus were similar to the Taura syndrome disease caused by the wild type TSV. The recombinant TSV was transmitted efficiently among healthy shrimp as demonstrated by River's postulates. Transcription mapping of the 5' end of the TSV transcript revealed that the virus maintained xxx vector-derived nucleotides at its 5' end. The maintenance of additional nucleotides at the 5' end of the TSV did not affect the infectivity of the virus in shrimp. The use of a baculovirus to express TSV in non-host cells opens avenues for investigating the replication of TSV and possibly other RNA viruses for which an immortal cell line is not available. In addition, the ability to produce the Taura syndrome disease via the injection of baculovirus containing a full-length TSV genome into shrimp is quite surprising, and opens up the possibility of delivery of RNA into shrimp cells via baculovirus mediated infection. To our knowledge this is the first report of the production of an infectious virus in marine shrimp using a non-host cell culture system.

## TILAPIA LAKE VIRUS AND ‘SUMMER MORTALITY’ SYNDROME IN EGYPT

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Egyptian fish farms have faced unexplained mortality of large tilapia during the summer months in recent years. Despite a number of researchers and organizations investigating potential causes, results so far have been inconclusive. However, a new orthomixovirus, Tilapia Lake Virus (TiLV) was identified in Israel in 2014 which was implicated in significant losses of tilapia in lakes and fish farms. More recently, the same virus has been identified in Columbia and Ecuador. This study aimed to determine whether TiLV is also present in Egypt, which shares a land border with Israel and whether, if present, TiLV is linked to ‘summer mortality’ of tilapia.

An epidemiological survey of 102 Egyptian pond-based tilapia monoculture and tilapia-mullet polyculture fish farms indicated that 38.3% of farms were affected by ‘summer mortality’ in 2015 rising to 47.1% in 2016. In polyculture farms, the mortalities only affect tilapia. Average mortality rates were estimated at 10.87% in 2015 and 15.71% in 2016 indicating that the problem is increasing year-on-year. The potential economic impact was estimated to be at least \$100 million per year.

Tissue samples from seven farms affected by ‘summer mortality’ were tested at the University of Stirling for TiLV. Samples from three farms tested positive using PCR; the first time that TiLV has been identified in Egypt. Sequencing yielded a TiLV sequence with 93% homology to published data on the Israeli strain of TiLV. Initial attempts to culture the virus were unsuccessful but will continue in 2017, to be followed by challenge trials to determine whether the Egyptian strain of TiLV is the causative agent behind ‘summer mortality’ of tilapia in Egypt.

Field research is continuing to build understanding of the epidemiological links between TiLV and ‘summer mortality’ and to develop local diagnostic capacity for consistent identification of TiLV from field samples. Work in 2015 and 2016 indicated that higher stocking densities and polyculture with mullet appear to be linked to increased prevalence of summer mortality while the disease patterns also suggests that surviving fish have resistance. Meanwhile best management practice recommendations also need to take into account this significant threat to African aquaculture.

# EFFECTS OF GILTHEAD SEABREAM *Sparus aurata* FISH FEED REPLACEMENT OF FISH MEAL AT DIFFERENT RATES USE OF CANOLA MEAL *Brassica spp* ON GROWTH RATE, FEED UTILISATION AND DIGESTIBILITY

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In this study was used of fish feed replacement of fish meal at different rates use of canola meal *Brassica spp.* in gilthead seabream *Sparus aurata* average of live weight  $49.86 \pm 0.01$ g 50 fish per tank and total 700 fish. In trial the feed nutrition value isonitrogen (45% protein) and isoncaloric (18% lipid). In this trial groups were designed the fish meal instead of using canola meal 0% (C0), 10% (CaM10), 15% (CaM15), 20% (CaM20), 25% (CaM25) and 30% (CaM30) and fish growth performance, feed conversion ratio and digestibility. At the end of the experiment the best weight gain in the control group ( $79.21 \pm 2.25$ g) as a result of the analysis showed that despite insignificant differences were found between groups ( $p > 0.05$ ). To the control group ordinary, respectively CaM30 ( $77.89 \pm 3.34$ g), CaM10 ( $77.29 \pm 2.26$ g), CaM25 ( $77.15 \pm 1.99$ g), CaM20 ( $76.46 \pm 2.66$ g) and CaM15 ( $75.84 \pm 2.84$ g) groups were followed. Specific growth rate also were similar trends. Feed conversion ratio (FCR) the lowest value in CaM30 group ( $1.54 \pm 0.1$ ) and the highest value in CaM10 group ( $2.31 \pm 0.2$ ); about of protein efficient rate the highest CaM30 group ( $1.52 \pm 0.01$ ) an the lowest CaM10 group ( $1.44 \pm 0.02$ ) were obtained and between the groups significant ( $p < 0.05$ ). Trial in the second phase of nutrients digestibility performance tries to determine dry matter and crude protein digestibility in the control group at the highest level while the lowest KA10 treatment group were determined and the difference between groups were significant.

Table 1 Biometric growth performance and digestibility values in trial groups ( $\bar{X} \pm SD$ ) \*

|            | C0                      | CaM 10                 | CaM 15                  | CaM 20                  | CaM 25                 | CaM 30                 |
|------------|-------------------------|------------------------|-------------------------|-------------------------|------------------------|------------------------|
| <b>IBW</b> | 49,87±0,01              | 49,86±0,01             | 49,86±0,01              | 49,86±0,01              | 49,86±0,01             | 49,86±0,01             |
| <b>FBW</b> | 79,21±2,25              | 77,29±2,26             | 75,84±2,84              | 76,46±2,66              | 77,15±1,99             | 77,89±3,34             |
| <b>PWG</b> | 58,84±4,51              | 55,01±4,55             | 52,10±5,68              | 53,35±5,36              | 54,74±4,03             | 56,2±6,71              |
| <b>SGR</b> | 0,47±0,03               | 0,44±0,03              | 0,42±0,04               | 0,43±0,04               | 0,44±0,03              | 0,45±0,04              |
| <b>FCR</b> | 1,80±0,5 <sup>ab</sup>  | 2,31±0,2 <sup>a</sup>  | 2,27±0,3 <sup>a</sup>   | 2,08±0,3 <sup>ab</sup>  | 1,60±0,2 <sup>b</sup>  | 1,54±0,1 <sup>b</sup>  |
| <b>PER</b> | 1,49±0,05 <sup>ab</sup> | 1,44±0,02 <sup>b</sup> | 1,47±0,03 <sup>ab</sup> | 1,48±0,03 <sup>ab</sup> | 1,51±0,02 <sup>a</sup> | 1,52±0,01 <sup>a</sup> |
| <b>DCP</b> | 95,7±1,4 <sup>a</sup>   | 88,0±0,3 <sup>d</sup>  | 91,2±0,2 <sup>bc</sup>  | 92,3±0,01 <sup>b</sup>  | 90,2±0,6 <sup>c</sup>  | 89,9±0,4 <sup>c</sup>  |
| <b>DCL</b> | 91,5±3,2 <sup>ab</sup>  | 83,4±1,7 <sup>d</sup>  | 89,4±0,6 <sup>ab</sup>  | 87,9±0,1 <sup>bc</sup>  | 85,0±0,7 <sup>cd</sup> | 92,9±0,1 <sup>a</sup>  |
| <b>DDM</b> | 54,4±2,7 <sup>a</sup>   | 41,9±2,7 <sup>b</sup>  | 53,0±4,1 <sup>a</sup>   | 54,0±4,0 <sup>a</sup>   | 44,8±4,4 <sup>b</sup>  | 45,8±2,3 <sup>b</sup>  |

Initial Body Weight (g) IBW, Final Body Weight (g) FBW, Percentage Weight Gain (%) PWG, Specific Growth Rate (%  $\text{day}^{-1}$ ) SGR, Feed Conversion Ratio ( $\text{g feed g day}^{-1}$ ) FCR, Protein Efficiency Rate PER, Digestibility Crude Protein (%) DCP, Digestibility Crude Lipid (%) DCL, Digestibility Dry Matter (DDM).

\*Values in the same row with different superscript letters are significantly different ( $P < 0.05$ ) from each other.

# EFFECTS OF GILTHEAD SEABREAM *Sparus aurata* FISH FEED REPLACEMENT OF FISH MEAL AT DIFFERENT RATES USE OF CANOLA MEAL *Brassica spp* ON GROWTH RATE, FEED UTILISATION AND DIGESTIBILITY

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In this study was used of fish feed replacement of fish meal at different rates use of canola meal *Brassica spp.* in gilthead seabream *Sparus aurata* average of live weight  $49.86 \pm 0.01$ g 50 fish per tank and total 700 fish. In trial the feed nutrition value isonitrogen (45% protein) and isoncaloric (18% lipid). In this trial groups were designed the fish meal instead of using canola meal 0% (C0), 10% (CaM10), 15% (CaM15), 20% (CaM20), 25% (CaM25) and 30% (CaM30) and fish growth performance, feed conversion ratio and digestibility. At the end of the experiment the best weight gain in the control group ( $79.21 \pm 2.25$ g) as a result of the analysis showed that despite insignificant differences were found between groups ( $p > 0.05$ ). To the control group ordinary, respectively CaM30 ( $77.89 \pm 3.34$ g), CaM10 ( $77.29 \pm 2.26$ g), CaM25 ( $77.15 \pm 1.99$ g), CaM20 ( $76.46 \pm 2.66$ g) and CaM15 ( $75.84 \pm 2.84$ g) groups were followed. Specific growth rate also were similar trends. Feed conversion ratio (FCR) the lowest value in CaM30 group ( $1.54 \pm 0.1$ ) and the highest value in CaM10 group ( $5.31 \pm 0.2$ ); about of protein efficienct rate the highest CaM30 group ( $0.52 \pm 0.01$ ) an the lowest CaM10 group ( $0.44 \pm 0.02$ ); about of productive protein value the highest CaM30 group ( $0.318 \pm 0.02$ ) and the lowest CaM25 group ( $0.185 \pm 0.07$ ) were obtained and between the groups significant ( $p < 0.05$ ). Trial in the second phase of nutrients digestibility performance tries to determine dry matter and crude protein digestibility in the control group at the highest level while the lowest KA10 treatment group were determined and the difference between groups were significant.

Table 1 Biometric growth performance and digestitibility values in trial groups ( $\bar{X} \pm SD$ ) \*

|            | C0                   | CaM 10            | CaM 15               | CaM 20               | CaM 25              | CaM 30            |
|------------|----------------------|-------------------|----------------------|----------------------|---------------------|-------------------|
| <b>IBW</b> | $49,87 \pm 0,01$     | $49,86 \pm 0,01$  | $49,86 \pm 0,01$     | $49,86 \pm 0,01$     | $49,86 \pm 0,01$    | $49,86 \pm 0,01$  |
| <b>FBW</b> | $79,21 \pm 2,25$     | $77,29 \pm 2,26$  | $75,84 \pm 2,84$     | $76,46 \pm 2,66$     | $77,15 \pm 1,99$    | $77,89 \pm 3,34$  |
| <b>PWG</b> | $58,84 \pm 4,51$     | $55,01 \pm 4,55$  | $52,10 \pm 5,68$     | $53,35 \pm 5,36$     | $54,74 \pm 4,03$    | $56,2 \pm 6,71$   |
| <b>SGR</b> | $0,47 \pm 0,03$      | $0,44 \pm 0,03$   | $0,42 \pm 0,04$      | $0,43 \pm 0,04$      | $0,44 \pm 0,03$     | $0,45 \pm 0,04$   |
| <b>FCR</b> | $1,80 \pm 0,5^{ab}$  | $2,31 \pm 0,2^a$  | $2,27 \pm 0,3^a$     | $2,08 \pm 0,3^{ab}$  | $1,60 \pm 0,2^b$    | $1,54 \pm 0,1^b$  |
| <b>PER</b> | $1,49 \pm 0,05^{ab}$ | $1,44 \pm 0,02^b$ | $1,47 \pm 0,03^{ab}$ | $1,48 \pm 0,03^{ab}$ | $1,51 \pm 0,02^a$   | $1,52 \pm 0,01^a$ |
| <b>DCP</b> | $95,7 \pm 1,4^a$     | $88,0 \pm 0,3^d$  | $91,2 \pm 0,2^{bc}$  | $92,3 \pm 0,01^b$    | $90,2 \pm 0,6^c$    | $89,9 \pm 0,4^c$  |
| <b>DCL</b> | $91,5 \pm 3,2^{ab}$  | $83,4 \pm 1,7^d$  | $89,4 \pm 0,6^{ab}$  | $87,9 \pm 0,1^{bc}$  | $85,0 \pm 0,7^{cd}$ | $92,9 \pm 0,1^a$  |
| <b>DDM</b> | $54,4 \pm 2,7^a$     | $41,9 \pm 2,7^b$  | $53,0 \pm 4,1^a$     | $54,0 \pm 4,0^a$     | $44,8 \pm 4,4^b$    | $45,8 \pm 2,3^b$  |

Initial Body Weight (g) IBW, Final Body Weight (g) FBW, Percentage Weight Gain (%) PWG, Specific Growth Rate (%  $\text{day}^{-1}$ ) SGR, Feed Conversion Ratio ( $\text{g feed g day}^{-1}$ ) FCR, Protein Efficiency Rate PER, Digestibility Crude Protein (%) DCP, Digestibility Crude Lipid (%) DCL, Digesitibility Dry Matter (DDM).

\*Values in the same row with different superscript letters are significantly different ( $P < 0.05$ ) from each other.



## EFFECT OF MEDICINAL PLANTS OF *Origanum onites* AND *Origanum vulgare* ESSENTIAL OIL ON THE LYSOSYME ACTIVITY IN RAINBOW TROUT *Oncorhynchus mykiss*

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Infectious diseases represent the main problem for the development and sustainability of the aquaculture industry as they cause significant economic losses as they restrict productivity and require the use of control measures that are often very expensive. However, the indiscriminate administration of antibiotics or other drugs in fish leads to the selection of antibiotic-resistant bacterial strains as well as to the accumulation of chemical residues in water and fish tissues, which may prove damaging to the environment and be potentially dangerous for consumers.

The research was aimed to determine the effect of different increasing levels of *Origanum onites* and *Origanum vulgare* essential oil as feed additives in rainbow trout diets in order to observe their influence in serum lysozyme activity.

Results showed that the levels of lysozyme activity in different experimental concentrations, 1.5, 2.5, 3.0 mL essential oil per kg of diet, had significantly higher compared to control. in *O. vulgare* after 60 days ( $P < 0.05$ ).

Lysozyme activity was significantly higher in rainbow trout fed diet containing 3.0 mL essential oil per kg of diet than other treatments and control after 60 days in *O. onites* ( $P < 0.05$ ). This results suggested that the essential oil of *O. onites* and *O. vulgare* increase lysozyme activity and also may improve disease resistance to pathogens when added to rainbow trout feed.

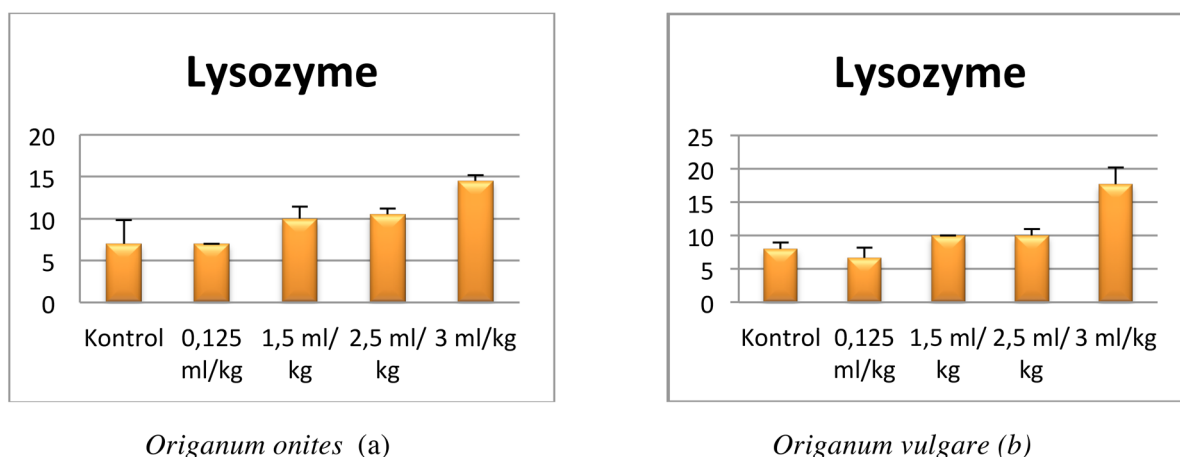


Fig 1. Lysozyme activity in different experimental concentrations *O.onites* (a) and *O. vulgare* (b)

## PROTEIN REQUIREMENT OF PACIFIC WHITE SHRIMP AT THE INITIAL REARING PHASE IN BIOFLOC SYSTEM

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This study evaluated the dietary protein requirement for Pacific white shrimp in biofloc system during the initial phase of rearing, as well as the effect of these diets on water quality and growth performance parameters. During the rearing period, fifteen circular tanks (400 L) were maintained in an isolated room with water heating system and constant aeration. Shrimps were fed using five practical diets, formulated with the increasing crude protein (CP) levels 31.28; 36.29; 41.57; 46.34 and 51.74 g 100g<sup>-1</sup>. Post-larvae (PL) (0.16 ± 0.01 g) were reared for 38 days at stocking density of 450 PL m<sup>-3</sup> in a completely randomized design. All the water quality parameters remained within the appropriate limits for rearing. Shrimp fed diets containing 31.28 to 46.34 g 100g<sup>-1</sup> CP had increasing final weight (1.52 - 2.61 g), yield (0.69 - 1.10 kg m<sup>-3</sup>), weight gain (1.38 - 2.44 g) and feed efficiency (77.28 - 101.68 %), which, on the other hand, decreased in 51.74 g 100g<sup>-1</sup> CP treatment (P<0.05) (Table 1). Mean survival was over 80%, with no difference (P>0.05) between treatments (Table 1). In conclusion, the range between 44.26 and 47.12 g 100g<sup>-1</sup> PB provided better growth performance of *L. vannamei* at the initial phase of rearing in biofloc system.

Table 1. Shrimp (*L. vannamei*) performance reared in biofloc system after 38 days fed with diets contain different levels of crude protein (shrimp initial weight 0.16±0.01 g).

|                                       | Crude protein g 100g <sup>-1</sup> |              |              |              |              | Quadratic effect   |
|---------------------------------------|------------------------------------|--------------|--------------|--------------|--------------|--|
|                                       | 31.28                              | 36.29        | 41.57        | 46.34        | 51.74        |  |
| Final weight<br>(g)                   | 1.52±0.19                          | 2.01±0.66    | 2.28±0.19    | 2.61±0.63    | 2.35±0.40    | y = -0.003x <sup>2</sup> +0.302x-<br>5.035<br>R <sup>2</sup> = 0.951 |
| Survival<br>(%)                       | 93.89±8.64                         | 90.37±8.91   | 92.59±4.79   | 94.44±7.86   | 84.63±12.25  | No significant   |
| Weight gain<br>(g)                    | 248.92±63.28                       | 292.62±76.06 | 349.97±24.79 | 409.89±68.90 | 327.58±68.13 | y = -0.514x <sup>2</sup> +49.05x-<br>802.9<br>R <sup>2</sup> = 0.761 |
| Productivity<br>(Kg m <sup>-3</sup> ) | 0.69±0.16                          | 0.80±0.19    | 0.95±0.06    | 1.10±0.17    | 0.89±0.17    | y = -0.001x <sup>2</sup> +0.124x-<br>1.974<br>R <sup>2</sup> = 0.763 |

All coefficients are significant by ANOVA (p<0.05).

## ESTABLISHING THE BASELINE OF SEA CUCUMBER AQUACULTURE IN EUROPE

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Sea cucumbers from the Mediterranean Sea, are being commercially exploited in Turkey since 1996. During the last years, there has also been a development of sea cucumber harvest in another countries from this area and NE Atlantic, in spite of the lack of specific legislation for their fisheries or poor regulation. This situation becomes to negative effects on wild populations such as loss of the biggest individuals and decrease of density, abundance and genetic diversity.

Under this current scenario, the development of aquaculture biotechnology for these new target sea cucumber species could play an important role to decrease the pressure on wild populations and supply the high demand from the Asiatic market. In Europe, the only species successfully produced for aquaculture purpose, is *Holothuria arguinensis*. Its embryonic and larval development has been already described and its juveniles produced and maintained; being their growth followed during the last three years. Important improvements have been achieved specially on its settlement survival and rearing conditions. Additionally, the first attempts to produced *H. mammata* have been done, obtaining the first juveniles.

Development of adequate and specie-specific rearing conditions in aquaculture is needed to ensure the health and welfare of sea cucumbers. Experiment to assess optimal diet for improving growth on *H. arguinensis*' adults was performed. It was tested two experimental diets based on seagrass debris, *Zostera noltii* and *Cymodocea nodosa*, with two levels of seagrass incorporated to the diet (15% and 40% of tank biomass). *H. arguinensis* showed higher growth ( $SGR = 0.1 \pm 0.06 \text{ \% d}^{-1}$  and  $\%WC = 5.86 \pm 3.57 \text{ \%}$ ) when it was feed with *Z. noltii*. Therefore, under tank conditions, *Z. noltii* seems to be an adequate diet supplement to improve growth in adults.

The optimal stocking density for *H. arguinensis* in tanks was also assessed. Four different stocking densities were tested (5, 15, 25 and 35 individuals/m<sup>2</sup>) and the growth (SGR and %WC) was evaluated during two months. Individuals under the stocking density of 5 ind/m<sup>2</sup> showed a significant higher growth rate ( $SGR = 1.25 \pm 0.08 \text{ \% d}^{-1}$  and  $\%WC = 104.66 \pm 9.98 \text{ \%}$ ) than the other ones. Therefore, to ensure growth and welfare of *H. arguinensis* under tanks conditions, lower sea cucumber biomass of 525.25 g/m<sup>2</sup> should be maintained, it could be considered as critic biomass.

Additionally, *H. mammata* aquaculture is being developed and its early developmental stages have been already described. Optimal density of this species for maintenance in tanks has been also tested and in the following months, diets improving its growth would be assessed.

## DEFORMITIES ELONGATION OF THE LOWER JAW IN JUVENILES YELLOWTAIL KINGFISH *Seriola lalandi* ARE ASSOCIATED WITH AN EXACERBATED EXPRESSION OF GENES PATHWAYS RELATED WITH FATTY ACID METABOLISM

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*Seriola lalandi* is a teleost fish that is distributed along the coasts of the Pacific and Atlantic Ocean. This species has shown a high potential for its use in aquaculture. However, one of the main constraints is the unpredictable level of skeletal deformities of the mandibular type. Nutritional factors such as n-3 PUFA have been related to influence the occurrence of bone malformations, but yet studied in further detail. In this paper, we are aimed at analyzing the transcriptional factors related with skeletal deformities at the larval stage.

Larvae were provided by Acuinor S.A., a commercial company that has successfully developed the complete production in captivity in a hatchery production center located in Caldera, Atacama Region, Chile. Two groups of juveniles with 40 days' post-hatch from different spawns of the same batch of broodstocks were collected in the spawning period and reared in different tanks until 42 days. In general, the percentage of deformities in the different batches was relatively low and samples were obtained from normal and deformed jaw individuals. RNA was obtained from the lower jaw and liver. A total of 16 libraries were obtained from pools of 4 individuals using the Illumina MiSeq. Filtered reads were mapped to a reference transcriptome obtained *de novo*, and the differential expression was then analyzed using EDGER, using a false discovery rate of 5%. Comparisons of annotated transcripts in the jaw revealed that 1387 were up-regulated and 58 down-regulated genes in the deformed larvae, suggesting an exacerbated gene expression of lipid metabolism and plasma lipid transport, which are important for bone remodeling, increasing the rate of bone formation, but bone absorption appear to be unaffected. The liver showed no transcripts expressed differentially. A list of the most significant transcripts related with different processes is presented in table 1. Our results suggest that deformities are a rather local process which is mainly related with and increase gene expression of genes related to pathways supporting bone formation, but not bone absorption. This is expressed at the phenotypic level, as a hyperplasia of the cartilage of the lower jaw. This dysregulation of bone formation is likely due to differences in availability of DHA in the bone itself, and we are further analyzing to what extent this phenotype can be predicted based on the availability of fatty acids in situ using explants. To our knowledge, this is the first study aimed at analyzing this complex phenotype in *Seriola lalandi*.

Table 1. Candidate genes from up-regulated in deformed larvae

| Candidates genes                        | Process involved                     |
|---|--------------------------------------|
| ApoA-IV, ApoE, Apob100, ApoA-I, ApoC-II | Plasma lipid transport               |
| FABP1, FATP                             | Proteins transporters of fatty acids |
| ACSBG2                                  | Synthesis of cellular lipids         |
| LPL                                     | Hydrolysis of lipoproteins           |
| IGF-1, Frizzled-5                       | Pathways signaling of Wnt            |
| MMP                                     | Pathways signaling of TNF            |
| IL-1, IL-6                              | Osteoclastogenic cytokines           |
| Col1a2, ITGb1                           | ECM component                        |

## EFFECT OF PARTIAL REPLACEMENT OF FISH MEAL BY GRADED LEVELS OF YEAST-FERMENTED RAPESEED MEAL ON GROWTH, INNATE IMMUNE RESPONSES AND OXIDATIVE STRESS OF JUVENILES RED SEA BREAM *Pagrus major*

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Rapeseed/Canola meal (CM) is a by-product of industrial oil extraction widely used in livestock feeding. CM has an excellent amino acids balance, but high levels of fibre and diverse anti-nutrients. Recently, much research has focused on the feeding value of CM/processed CM and their importance as a feed ingredient in Aquaculture. Solid state fermentation has been identified as the preferred method for the enrichment of CM since it offers several economical and practical advantages. Therefore, a 9-week feeding trial was conducted to evaluate the possible effects of Yeast (*Saccharomyces cerevisiae*) fermented CM (YFM) on growth, plasma metabolites, innate immune response and oxidative stress of juvenile red sea bream (initial average weight, 3.5 g).

Triplicate fish groups received five isonitrogenous and isocaloric diets with fish meal protein replacements of 0% (FM0), 18.75% (FM1), 37.5% (FM2), 56.25% (FM3) and 75% (FM4) respectively. The results indicated that only groups fed with FM4 diets showed significantly reduced ( $P = 0.05$ ) Final body weight, Weight gain, Specific growth rate, and Feed intake when compared with control groups. Whole body Protein and Lipid composition showed also similar trends. However, Feed conversion ratio, Protein efficiency ratio, and Survival were unaffected by any treatment. Blood Hematocrit level decreased progressively among dietary treatments but only FM4 fed group showed significant differences ( $P = 0.05$ ) compared with control group. Blood parameters (Glucose, Cholesterol, Blood Urea Nitrogen, Bilirubin, Triglyceride, Total serum protein) and innate immune response parameters (Lysozyme, Catalase and Peroxidase activities) were not altered by dietary treatments. But interestingly, Cholesterol level decreased with YFM inclusion. With regard to oxidative stress, FM1 and FM2 fed groups showed best condition with low oxidative stress and high antioxidant potential than control and other groups. Moreover, freshwater stress resistance was not affected by YFM inclusion among all the treatments.

Therefore, we can conclude that protein from YFM could substitute 56.25% fish meal protein without negative effects on growth, nutrients utilization, innate immune response and oxidative stress of juvenile Red sea bream.

TABLE 1. Diet formulation for Red sea bream

|       | Diet |      |      |      |      |
|-------|------|------|------|------|------|
|       | FM0  | FM1  | FM2  | FM3  | FM4  |
| FM    | 47   | 38.2 | 29.4 | 20.5 | 11.7 |
| YFM   |      | 14   | 28   | 42   | 55.8 |
| Other | 53   | 47.8 | 42.6 | 37.5 | 32.5 |

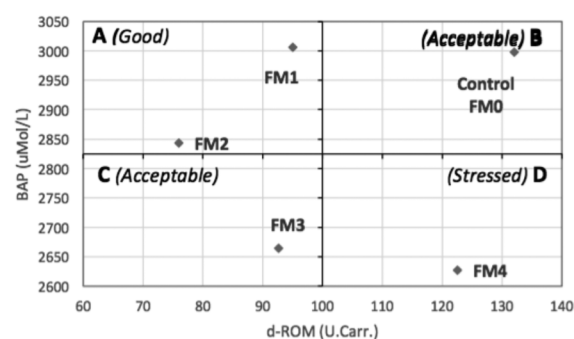


Fig 1. Oxidative stress parameters in Red sea bream (*P.major*) fed test diets for 65 days. Central axis based on mean values of d-ROM (Reactive oxygen metabolites) and BAP (Biological antioxidant potential).

## THE STORY OF THE FLY AND HOW IT COULD SAVE THE WORLD

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Delegates to World Aquaculture 2017 landing at Cape Town International Airport will have flown over what is by headcount the world's biggest farm: a fly-farm, in which an army of 8.5 billion flies is busily converting organic waste into high-protein feed ingredients for fish, poultry and pigs. A South African success story, the farm is a pioneer in the emerging waste-to-nutrient up-cycling industry, which is creating a new and sustainable source of protein to replace fishmeal in animal diets. There is an urgent need for this in aquaculture, as demand for fishmeal is predicted to outpace supply by 5 million tonnes pa by 2024.

Fly larvae are a natural source of protein for fish in the wild, unlike soya, and their chemical composition is almost exactly the same as that of fishmeal. The farm, operated by AgriProtein, produces fly larvae at a truly industrial scale. It takes unwanted organic waste from a variety of sources and feed this to eggs laid by its black soldier fly breeding stock. The eggs hatch into larvae and grow at an enormous rate: 1kg of fly eggs turns into 380kg of larvae in 72 hours. The farm produces nearly 5,000 tonnes of insect meal and 2,000 tonnes of lipids per year, saving 250 tonnes of organic waste per day from landfill. In the next 10 years, the company plans to roll out 200 more of these fly-farms globally under licence.

Waste-to-nutrient up-cycling delivers three benefits to the world. First, it helps solve the food security challenge. Replacing fish and soy protein in animal feeds frees up all of our oceans and more of our farmland to feed humans. Second, replacing fishmeal with insect meal allows the oceans to heal and reduces greenhouse gases at every stage of the fishmeal production chain from point-of-catch to point-of-sale. This is a key reason why environmentally-responsible aquaculture businesses are seeking to reduce their reliance on fishmeal as a feed. Third, using organic waste as a rearing material helps tackle the waste crisis. Organic waste makes up nearly one half of all solid municipal waste and is predicted to grow to 1 billion tonnes per annum by 2025. Finding a use for it reduces disposal costs, greenhouse gases, leachates and the need for extra landfill.

Waste-to-nutrient up-cycling is still in its early days, but supply and environmental imperatives will make it extremely important to aquaculture in the years to come. Delegates to World Aquaculture 2017 will gain an early insight into the contribution the humble fly will make to their industry and to the planet.

See also links to TED talks by Jason Drew:

<https://www.youtube.com/watch?v=T-RqnmXKhvc>

<https://www.youtube.com/watch?v=hxeuQtFbwZo>



## **ESTABLISHING THE SUITABILITY OF NANDONI DAM FOR THE CAGE CULTURE OF TILAPIA SPP.**

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The Aquaculture Research and Development component of the Department of Agriculture, Forestry and Fisheries investigated the suitability of Nandoni dam in terms of its physical, chemical and biological attributes as related to the requirements for tilapia culture. The Nandoni dam is situated in the northern region of South Africa, in the Limpopo Province on the Luvuvhu River which has its origin in the Soutpansberg Mountains. Field surveys commenced in May 2016 with follow up surveys in September, November, December and April 2017. Each survey included the sampling of a transect of 6 stations along the length of the dam. Profiles of conductivity, temperature and depth (CTD) were taken at each station using a SBE-19 Plus Seacat profiler. The Seacat was equipped with additional sensors: SBE 43 oxygen sensor, WETLabs ECO fluorometer (470/695 nm) and turbidity sensor (optical scattering at 700 nm), WETLabs C-Star transmissometer (660 nm), and Biospherical QSP PAR irradiance sensor. Several instruments including a Turner Designs <sup>TM</sup> C3 fluorometer, 4 Starmon mini <sup>TM</sup> temperature loggers and 2 JFE Advantech RINKO<sup>TM</sup> dissolved oxygen sensors were deployed at specified depths near the dam wall (40 m depth) to provide continuous records. Two water quality parameters are likely to provide a suboptimal environment for the culture of tilapia. Although surface water temperatures of around 25 °C for much of the year may be suitable for tilapia, the cooling of the epilimnion during June, July and August to <20 °C winter is likely to reduce growth. A squeezing of suitable habitat is also likely during summer and autumn owing to severe depletion of oxygen in the thermocline. Consequently a sharp oxycline coincident with the thermocline and the establishment of a hypoxic/anoxic metalimnion/hypolimnion will further restrict viable finfish culture operations to the upper 5 m of the dam.



## TEACHING EXPERIMENTAL DESIGN, DATA ANALYSIS AND SCIENTIFIC WRITING AS PART OF THE THIRD-YEAR ICHTHYOLOGY CURRICULUM AT RHODES UNIVERSITY, SOUTH AFRICA

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Students in their second and third year obtain knowledge mostly from textbooks, but until that time they seldom had the opportunity to participate in the scientific process that generated such information. I report on my experience of teaching third-year students in ichthyology and fisheries science the methods to obtain knowledge as a result of conducting a scientific investigation. The students learn to make a switch from exercising an assigned task, for example, a given project with exact instructions, to owning knowledge. In this context, ownership results from the fact that the students design an experiment as a small group and that they need to defend their chosen methods to their peers. In my experience, this process can be taught at third-year level and the work has resulted in publications in scientific journals.

This eight-week course consists of four sections. In the first two weeks, there are lectures and tutorials on the basic concepts of experimental design and methods of data analysis. This is followed by a period during which the students conduct pilot experiments under guidance. Based on their experience from these initial studies, we discuss how to improve the experimental design and the methods. Topics include randomisation, replication, sample size determination, pseudoreplication bias, hypotheses-driven research concepts, data analysis and interpretation of results.

Initially, each group of 3–4 students presents a research proposal to their peers. This is followed by discussions, fostering independent thinking and fair criticism. These discussions provide input for the writing of a research proposal. Once this proposal has been approved, the students conduct the main experiment over the following 2–3 weeks. We then analyse and interpret the data together in small groups and prepare a scientific manuscript. At the end of the course, each group presents a seminar with methods, results and a discussion.

When the data that need to be analysed are *owned* by the student as a result of an experiment that they have conceptualised, there is usually an interest in data analysis and interpretation. I suggest that the success of teaching experimental design, data analysis and scientific writing depends to some extent on the level of ownership of the information obtained during the research.

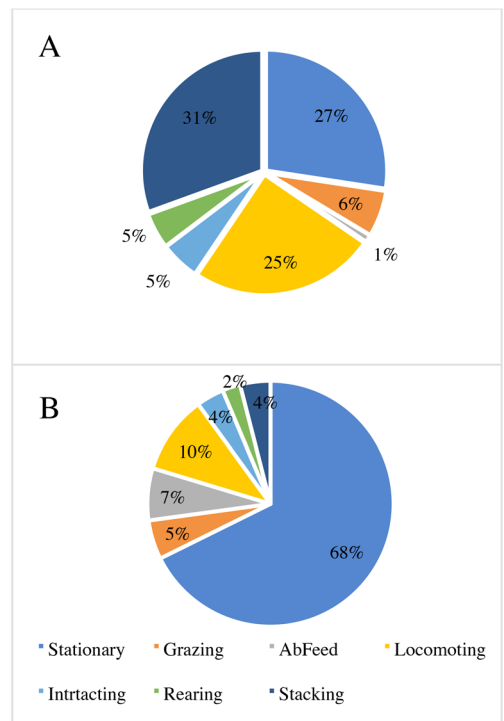


Figure 1: The proportion of time spent by *Haliotis midae* at night on seven state behaviours at stocking densities of 24% (A) and 8% (B).

## ANAESTHESIA IN FISH AFTER PRE-DOSING WITH THE ANAESTHETIC AT A LOW CONCENTRATION FOR A SHORT PERIOD

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Anaesthesia in fish has been achieved with a wide range of methods and compounds, generally with the aim of rapid induction of anaesthesia followed by fast recovery. Many experiments on the use of anaesthetics have been designed to estimate induction time and recovery time as a function of the concentration of the chemical used.

We report on a new technique of using a range of anaesthetics in several fish species. Fish were initially exposed to a low non-sedative concentration of the selected anaesthetic for a short time of up to three minutes. This was followed by exposure to a concentration that was known to safely anaesthetise the species. Thus, experiments were designed to 1) expose fish briefly to a low concentration with the rest of the dosage being added after a few minutes, or 2) to expose fish to the full concentration for the same amount of time (control treatment). The product of concentration and duration of exposure was kept the same between treatments so that only the method of application could be tested. Time to induction to anaesthesia, time to recovery and behavioural responses were recorded. Water quality (pH and dissolved oxygen) and water temperature, as well as size and sex of the fish were used as covariates in all analyses.

Guppies (*Poecilia reticulata*) exposed to clove oil ( $60 \mu\text{g L}^{-1}$ ) using the pre-dosing technique ( $8 \mu\text{g L}^{-1}$  followed by  $52 \mu\text{g L}^{-1}$ ) recovered 36% faster from anaesthesia than control fish. In rainbow trout (*Oncorhynchus mykiss*) recovery time after a short exposure to a low concentration of clove oil (15% of the total concentration) could be improved by up to 27%. In mosquito fish (*Gambusia affinis*) the results differed between anaesthetics. Pre-exposure to 2-phenoxyethanol could reduce recovery time by 25%, while this effect could not be shown when using clove oil. In tilapia (*Oreochromis mossambicus*) pre-exposure to benzocaine hydrochloride may reduce recovery time.

Results varied between species and the anaesthetic used. In some cases, pre-dosing was beneficial, but this could not be shown in all experiments. In addition, the sex of the fish and water pH significantly influenced both time to reach anaesthesia and recovery times.

Since both duration of exposure (S) and concentration (C) could influence recovery time, we suggest using the product of these two variables (S x C) to compare results from experiments and to standardise units. Although benefits of pre-dosing could not be demonstrated under all conditions, some results provided a motivation to test this method in other species and with different anaesthetics.

## THE USE OF CRYSTAL MENTHOL AS AN ANAESTHETIC IN TILAPIA *Oreochromis mossambicus* AT THREE WATER SALINITIES AND TWO TEMPERATURES

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Fish anaesthesia has been used for a wide range of applications, such as weighing and measuring of fish, fish transport, or surgical procedures. We report on the first use of crystal menthol as an anaesthetic in juvenile tilapia *Oreochromis mossambicus* at three water salinity levels and two temperatures.

To test the effect of water salinity on average induction and recovery times, fish were exposed individually ( $n = 20$  per treatment) to  $85 \text{ mg L}^{-1}$  crystal menthol dissolved in ethanol at three salinity levels (0, 15 and 35 ppt). Fish in freshwater were anaesthetised significantly faster than those kept at the other two salinities, while the average recovery time did not differ between the three treatments. In the freshwater treatment, the average times (seconds  $\pm$  standard error) for induction and recovery were  $227 \pm 10.5 \text{ s}$  and  $326 \pm 28.0 \text{ s}$ , respectively. At the highest salinity, average times to achieve induction and recovery were  $343 \pm 16.8 \text{ s}$  and  $371 \pm 39.2 \text{ s}$ , respectively. These values were not significantly different to those recorded at the medium salinity ( $359 \pm 13.5 \text{ s}$  and  $367 \pm 35.9 \text{ s}$ , respectively). Average fish mass ( $2.81 \pm 0.18 \text{ g fish}^{-1}$ ,  $n = 60$ ) and length ( $56 \pm 2.3 \text{ mm}$ ,  $n = 60$ ) did not significantly influence results when used as covariates. There was no significant correlation ( $p > 0.05$ ) between time to anaesthesia and time to recovery within each of the three treatments.

In a second experiment, the effect of temperature on induction and recovery time was tested by exposing fish to  $85 \text{ mg L}^{-1}$  crystal menthol at  $21^\circ\text{C}$  and  $27^\circ\text{C}$  in freshwater ( $n = 30$  fish per treatment). Temperature significantly influenced time to anaesthesia and recovery ( $p < 0.001$ ). At  $27^\circ\text{C}$ , fish were anaesthetised within  $340 \pm 21 \text{ s}$ , which was 110 seconds faster than at  $21^\circ\text{C}$  ( $450 \pm 39 \text{ s}$ ). Fish at the higher temperature recovered significantly faster ( $251 \pm 15 \text{ s}$ ) than fish at  $21^\circ\text{C}$  ( $316 \pm 12 \text{ s}$ ) even though they had been exposed to the anaesthetic for the same duration. There was no correlation between fish mass ( $2.6 \pm 0.1 \text{ g fish}^{-1}$ ,  $n = 60$ ) and length ( $56 \pm 0.8 \text{ mm}$ ,  $n = 60$ ) and time to anaesthesia or time to recovery ( $p > 0.05$ ) within the two treatments.

In both experiments, there were no mortalities. Crystal menthol could thus be considered for use as an anaesthetic in this species at a wide range of salinity values and temperatures as it achieved fast anaesthetic induction times and reliable recovery.

## THE TAQUILLA CLAM, *Mulinia edulis*, ADVANCES IN OPTIMIZATION OF THE CULTURE TECHNOLOGY

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The world demand for clams is growing, and its market is currently estimated at 1 billion dollars. There is also a select demand for small size clams (3cm) in Chile, Latin America, Europe, and North America, which is expected to increase four wise in the following 15 years.

The aim of this paper is to show the advances in technology optimization in the different culture steps in *Mulinia edulis*. In order to obtain an optimized technology, the following experiments have been done: (1) Larval culture: Culture densities, diet, food ration and culture systems; (2) Postlarval culture: Culture densities, diet, food ration and culture systems (with sand and down-welling); (3) Early grown out: culture density, with and without substrate in suspended systems; (4) Grown-out: Rearing systems in the intertidal zone (with and without predator protection), culture density and season of seed planting.

In the optimized larval cultures, the D larvae are cultured in 1000 l tanks at a density of 10 larvae/ml and fed with 25000 cel\*ml\*day<sup>-1</sup> of a mixed diet of microalgae. After 20 days of culture we get pediveliger larvae of 280 µm with a survival rate of 40%. The pediveliger are cultured at a density of 10 larvae/cm<sup>2</sup> with an increasing ration of a mixed diet of microalgae; they reach a size of 2700µm in 36 days, with a survival rate of 60%. The early grown-out stage is made in a suspended system with sand up to 7mm and the bottom grown-out stage is made in the lower intertidal zone in plastic boxes with sand, covered with a mesh for protection from predators. Between the 8<sup>th</sup> and 12<sup>th</sup> month it's possible to harvest small size clams (3cm) with a survival rate of about 85% in clams cultured in the intertidal zone in Chiloé Island. The whole process, from the spawning to harvest, elapses between 12 to 15 months and its survival rate in average is 10% (See Table 1).

Currently, the industry in Chile only exports clams as a low price commodity to markets in Southern and Western Europe, with sizes over the legal minimum (5.5 cm). We expect that in the future, the Taquilla clam can be recognized in the market as a green certified product

Tabla 1: Optimization of culture of taquilla clams

|                     | Culture     | Size   | Density              | Duration Days | Survival |
|---------------------|-------------|--------|----------------------|---------------|----------|
| Hatchery            | DLarvae     | 80 µ   | 10/ml                | 20            | 40%      |
| Nursery             | Pediveliger | 280 µ  | 10/cm <sup>2</sup>   | 36            | 60%      |
| Suspended Grown-out | Early seeds | 2700 µ | 2/cm <sup>2</sup>    | 40            | 52%      |
| Bottom Grown-out    | Seeds       | 7 mm   | 1,450/m <sup>2</sup> | 270           | 80%      |
| Total               |             |        |                      | 366           | 10%      |

## IMPORT, QUARANTINE, AND DISEASE TESTING OF THE AFRICAN LONGFIN EEL *Anguilla mossambica* IN MAINE, USA

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Global trade in immature glass eels or elvers captured from the wild is an integral component of eel aquaculture. Consequently, in many regions of the world one or more non-native eel species are imported for stocking aquaculture farms. The two primary risks to native stocks from this activity are considered to be escapement and disease. While escapement can be minimized or even eliminated with readily implemented physical barriers, managing disease risk may require a period of quarantine and disease testing following import, specific to regional concerns and regulations. As part of a feasibility trial, immature African longfin eels (*Anguilla mossambica*) were imported into Maine USA and stocked into a closed-containment recirculating aquaculture system for quarantine while disease testing was carried out. During the quarantine period all effluent was filtered to retain particles greater than 50 $\mu$ m, followed by ultraviolet light disinfection at a dose exceeding 180 MJ/cm<sup>2</sup>. Effluent discharge rate was limited to less than 25 l/m, and solids were stored and disinfected in a closed tank for off-site disposal. Within 1 week of transport 150 glass eels were sampled for disease screening at a level sufficient to detect pathogens at an assumed 2% prevalence. Parasitology was conducted to screen for the swim bladder nematode *Anguillicola papernai*. Virology was conducted on CHSE, EPC, GF-1, EK-1, and SSN cell lines, with mandatory reporting of cytopathic effect. Molecular screening was used for detection of *Franciscella* sp. and for *A. papernai*. Preliminary results showed no presence of any pathogens of regulatory concern.



Figure 1. African longfin eels one-month post transport

## ADVANCE NURSERY SYSTEM FOR UNCONDITIONED SANDFISH *Holothuria scabra* JUVENILES

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Ocean nursery systems are the most cost effective systems for production of early juvenile *Holothuria scabra* in the Philippines. Juveniles reared in floating *hapas* (fine mesh nets) however are not sediment-conditioned and survival rates are very variable. Intermediate culture in sea pen with and without predator exclusion nets were tested to determine whether *in situ* sediment conditioning together with protection from predation can improve survival. This ocean nursery system for juvenile sandfish juveniles was evaluated in two sites in Northwestern Philippines using (small:  $4.9 \pm 0.4$  g and large:  $9.4 \pm 0.5$  g) unconditioned juveniles. In Bolinao pens were set up in areas with high and low chl-a content. The burying behavior of the juveniles was determined every three hours for 24 hours after seven days. This was done to evaluate differences in emergence pattern and survival of juveniles reared in high and low chl-a content. Sediment and other environmental parameters were monitored to determine factors which may influence the growth and survival of the sandfish juveniles.

Results in Anda showed that survival in the pens with the predator exclusion net was not significantly different from those without. In both treatments, survival of the larger juveniles ( $62.8 \pm 6.8\%$ ) was significantly higher than the small juveniles ( $29.8 \pm 6.2\%$ ) reared after 7 days. However, survival decreased after 30 days for both the large and small juveniles. The average weight of juveniles increased over the 30 day experimental period except for the smaller juveniles without the predator exclusion nets.

After seven days, juveniles exhibited the typical diel activity pattern. Majority of the juveniles reared in both treatments were emerged between 2100H to 2400H. However, juveniles reared in nets with lower sediment chlorophyll-a spent more time grazing on the surface. Notably, average survival was only 38.5 and 47% after 7 days in the pens with lower chl-a content and higher chl-a respectively. After 30 days, survival was significantly higher in pens with high chl-a content in Bolinao at  $26.8 \pm 7.43\%$  compared to only 7% in the low chl-a pens. Growth rates ( $0.4 \pm 0.03$  to  $1.1$  g day<sup>-1</sup>) and mean weights ( $15.3 \pm 0.93$ g) of sandfish was higher in juveniles reared in high chl-a treatment in Bolinao. Sediment chl-a content from both treatments in Bolinao decreased by day 30. The decreased in sediment chl-a content could be attributed to high grazing activity by the juveniles, although shading effect by the predator exclusion net cannot be discounted.

## GROWTH AND SURVIVAL OF NILE TILAPIA UNDER NURSERY CONDITIONS

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One of the major challenges facing the rapidly developing aquaculture industry in Ghana is the lack of well established nurseries that serve as intermediaries between the hatcheries and the grow-out fish farmers. Nurseries are a vital link in the aquaculture value chain that needs to be well developed in order to strengthen the industry and are also needed for expanded production. This study was done to evaluate the survival, feed conversion, and growth of Nile tilapia raised in ponds on high-protein commercial feeds from fry to fingerling sizes. Nile tilapia were stocked at 1.9g and raised in 1m X 1m hapas in a 900m<sup>2</sup> pond. Two factors each with three replicates were considered using a completely randomised design: 1) stocking density at 25, 50, 75, and 100/m<sup>3</sup>, and 2) Time (time-to-harvest) at 10, 20, 30, and 40 d. Fish were fed twice daily to satiation on a 45% protein commercial feed. Harvesting was done at the predetermined times and the following measures were determined: survival, growth, and FCR. Survival was relatively high between 67-92%, and neither the time-to-harvest nor stocking density had a significant effect on fingerling survival. FCR was good and growth was good; with average final weights on day 40 being 20.6g, 19.47g, 20.5g and 21.6g in the treatments with 25/m<sup>2</sup>, 50/m<sup>2</sup>, 75/m<sup>2</sup> and 100/m<sup>2</sup> respectively. The maximum stocking density does not appear to have been reached as the highest stocking density had the highest final average weight. There is a real potential to develop nurseries in Ghana.



## ASSESSING THE AUTHORSHIP GENDER GAP IN AQUACULTURE-RELATED PUBLICATIONS

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Examining authorship position in aquaculture allows for a better understanding of the status of women in the discipline, as authorship is an important factor in assessing professional success. A study by West et al. 2013 found that men predominate in the first and last author positions and women are underrepresented in single-authored papers in a review of more than eight million papers in the JSTOR corpus across disciplines in natural sciences, social sciences, and humanities. Other studies have assessed women authorship in other disciplines such as law and medicine, and found that a gender gap in published literature still persists.

This study applies the large sample size and methodology of West et al. 2013 to the broad discipline of aquaculture, and compares these results to gender authorship in the International Aquaculture Curated Database (IACD) – a compilation of 543 peer-reviewed publications supported by four long-term international aquaculture programs headquartered at Oregon State University. By verifying the IACD, possibilities open for social network analysis and other studies. Preliminary baseline analyses reveal that the percentage of women authors (13.8%) was similar for the JSTOR aquaculture subsample and the IACD (15.7%). Women are not well represented in either database, and remain underrepresented as authors in any position in aquaculture journals. For single-authored papers, the JSTOR aquaculture subsample shows women represent 11.0% of single-authored papers since 1913. The result of single authorship by women in the ICAD is 11.1% since 1990. Percentages of first and last authorship positions were also comparable for the publications in the IACD and JSTOR Aquaculture subsample at 14.4% and 15.8%, respectively for first authors, and 14.0% and 16.5%, respectively for last authors. First and last author results from the overall JSTOR Corpus for all fields were slightly higher than for the field of aquaculture at 19.2% and 19.6%, respectively.

The next steps for this work include putting results into the broader context of women's representation in academic and professional positions in the field of aquaculture. Learning how authorship has changed in the aquaculture discipline over the last few decades is critical for promoting gender equity for future aquaculture scholarship and the sustainability of the professional discipline.

## EVALUATING THE EFFECTS OF METHANOL-EXTRACTED *Basella alba* ON THE MASCULINIZATION OF NILE TILAPIA *Oreochromis niloticus* (Linnaeus, 1758)

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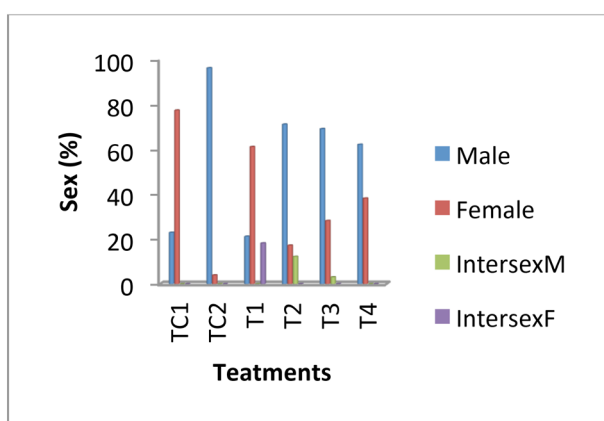
Naturally occurring sources of testosterone are alternative to 17 $\alpha$ -Methyltestosterone used for masculinizing Tilapia; since synthetic androgen is been criticized for possible health and environmental impacts such as hepatotoxicity and fetotoxicity in humans. Vine spinach, *Basella alba*, contains anabolic steroid, a potential source of dietary testosterone-enhancer. This study evaluated the effects of *B. alba* leaf extract on sex, growth, and survival rate of *Oreochromis niloticus*.

Newly hatched larvae of *Oreochromis niloticus* were collected from female broodstocks paired in the ratio 1 male: 3 females in hapas, using the natural breeding method. *O. niloticus* fry ( $8.57 \pm 0.01$  mg) stocked at 137fry/m<sup>2</sup> were fed with untreated diet (TC<sub>1</sub>), 60mg MT-treated diet (TC<sub>2</sub>), and methanol-extracted *Basella alba* (MEBA) at the rate 40(T<sub>1</sub>), 60(T<sub>2</sub>), 80(T<sub>3</sub>) and 100(T<sub>4</sub>) mgkg<sup>-1</sup>, for 28days. The survival percentage of fish in TC<sub>1</sub> and TC<sub>2</sub> was similar to those observed in the MEBA treated groups, but highest survival percentage ( $96.800 \pm 1.141\%$ ) was recorded in T<sub>3</sub>.

There was no significant difference ( $P > 0.05$ ) in the final weight gained by *O. niloticus*. Nevertheless, T<sub>2</sub> had the highest final weight and mean weight gain ( $13.325 \pm 2.864$ g and  $12.784 \pm 2.922$ g). All treatments, except T<sub>1</sub>, showed significantly higher ( $p < 0.05$ ) percentage of males. TC<sub>2</sub> had highest male percentage ( $95.650 \pm 0.707\%$ ); while amidst MEBA-treatment groups, T<sub>2</sub> had highest male and intersex male percentage ( $70.750 \pm 0.354\%$  and  $11.750 \pm 0.354\%$ ). TC<sub>1</sub> had highest female percentage ( $77.260 \pm 0.014\%$ ); while amidst MEBA-treatment groups, T<sub>1</sub> showed significantly higher ( $p < 0.05$ ) female and intersex female percentage ( $61.010 \pm 0.014\%$  and  $17.795 \pm 0.290$ ), as represented in figure 1 below.

Testosterone and estradiol levels were significantly highest ( $p < 0.05$ ) in T<sub>2</sub> (11.628 nmoL/L and 15.784nmoL/L).

The results from this study suggest that methanol-extracted *Basella alba* (MEBA) can be used to produce all-male tilapia population.



**Figure 1: Percentage of Sex of *Oreochromis niloticus* per Treatment**

## TILAPIA CULTURE SUCCESS IN EGYPT: A MESSAGE TO AFRICA

Abdel-Fattah M. El-Sayed

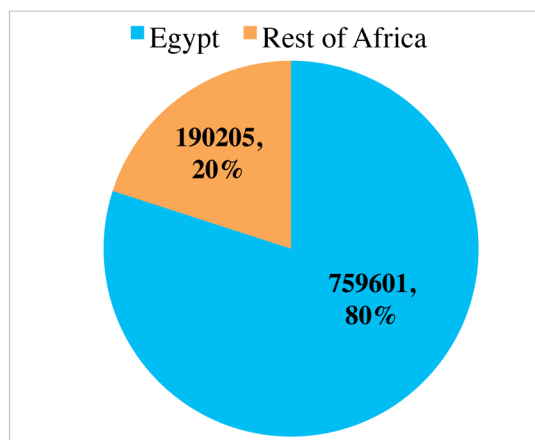
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Tilapia culture has traditionally been practiced in Egypt for thousands of years, while modern commercial tilapia culture started in late 1950s. Nile tilapia is the main cultured tilapia species in Egypt. Tilapia culture has been expanding at a progressive rate since the 1990s, with an annual growth rate of 16.5% during the past 10 years. As a result, the production of farmed tilapia in Egypt increased from 157,425 mt in 2000 to 759,601 mt in 2014, representing 67% of total national aquaculture production. Tilapia production in Egypt also represented 80% of total tilapia production in Africa in 2014. Egypt is currently ranked third, only after China and Indonesia, among the top tilapia producers in the world.

Nile tilapia are reared both semi-intensively in earthen ponds and intensively in cages, ponds and concrete tanks, and in integration with terrestrial crops. Tilapia monoculture and polyculture with carps and mullets are commonly practiced. Stocking densities vary significantly depending on pond size, depth, initial fish size and culture conditions. Organic and inorganic fertilizers are used for pond fertilization, in addition to supplemental feeding with 25-30% crude protein (cp) feeds. Manual feeding and demand feeders are commonly used for feeding farmed tilapia. The yield ranges from 5-9 mt/ha/cycle. Intensive culture of Nile tilapia in floating cages is also widely practiced. The fish are stocked in cages at a density of 60-100 fish/m<sup>3</sup> to yield 25-40 kg/m<sup>3</sup>. Intensive tilapia culture in earthen ponds, tanks and recirculating systems is slowly spreading in Egypt. Nile tilapia (2 to 20 g) are stocked in the ponds at a density of 50,000 to 100,000 fish/ha. The ponds and tanks are provided with aeration and formulated feeds. The fish grow to 200- >300 g in 5 to 8 months, yielding 15 to >20 mt/ha. Tilapia co-culture with land crops, such as rice and wheat crops, is also expanding.

Tilapia culture in Egypt is practiced mainly by the private sector, using simple farming technologies; with small farmers being the driving force of tilapia production. Therefore, the success of tilapia culture in Egypt can be easily transferred to, and adopted in, the rest of Africa. Taking advantage of the prevailing environmental conditions, locally available farming inputs and Egyptian experience can make tilapia culture in Africa a real success.



Production of farmed tilapia in Egypt in 2014.

## THE VALUE OF ZEBRAFISH (*Danio rerio*) AS AN INTEGRATIVE MODEL IN ECOTOXICOLOGY

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Bioassays play a basic role in different aspects of scientific approaches. Meanwhile, bioassays with zebrafish - an established model organism in different research areas - are increasingly being utilized in effect direct analysis. Aiming to contribute for the optimal application of zebrafish bioassays in effect direct analysis.

Over the recent decades, zebrafish bioassays have guided effect direct analysis of natural products and environmental samples. A vast majority of studies performed bioassays with embryos and early larvae, which allowed small-scale experimental setups. Furthermore, biotesting applied zebrafish methods in both screening phase as well as for further investigations. For dosing, several studies performed solvent exchange of extracts and fractions to dimethyl sulfoxide (DMSO) as carrier. However, high DMSO concentrations were required for the testing of complex matrix extracts, indicating that future studies might benefit from the evaluation of alternative carrier solvents or passive dosing.

Finally, a great advantage of zebrafish bioassays in effect direct analysis of environmental samples is the availability of mechanism- and endpoint-specific method for the identification of important classes of contaminants. The evaluation of estrogenic and non-estrogenic endpoints is considered to be a promising strategy to facilitate the identification of environmental contaminants and their impacts.

Confirming that, the action of alkylphenols (APs) on zebrafish (*Danio rerio*) embryos were assayed. Embryos were subjected to different doses of APs to study the effect of such class of chemicals on the fish embryogenesis during the first 120 hours of fertilization. General morphological abnormalities and teratogenicity score were analyzed.

## HEAVY METALS ASSESSMENT IN THE STRIPED VENUS CLAM *Chamelea gallina* IN EGYPTIAN FISHERIES AS POTENTIAL CANDIDATE FOR EXPLOITATION AND AQUACULTURE

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The striped Venus clam *Chamelea gallina* was suggested for the first time as potential candidate for exploitation from its natural fisheries and for possible aquaculture in Egypt. The present study was conducted to investigate the heavy metals concentrations in *Chamelea gallina* from natural fisheries to evaluate its safety for human consumption as potential bivalve resource for exploitation in Egypt.

*Chamelea gallina* was sampled from 12 transects representative of about 60 km along the Mediterranean coast between Rosetta and Burullus from 31.45874 N and 30.50054 E to 31.59596 N and 30.94383 E. Clam soft tissues in each of 24 samples (50 clams or less/sample according to availability) were examined for levels of eight heavy metals; four hazardous metals [lead (Pb), cadmium (Cd), Mercury (Hg) and chromium (Cr)] and four essential metals [copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn)]. SHIMADZU Atomic Absorption Spectrophotometer AA-6800 equipped with GTA furnace and GVA cold vapor unit as well as flame unit was used for the analysis. The Results were compared to authorities' maximum permissible limits as set by different organizations of different countries on both dry weight (d.w) and wet weight (w.w.) basis.

Results showed that the examined bivalve fisheries are considered clean compared to other areas of the world and are considered safe for human consumption considering FAO/WHO, USFDA and other regulations from different countries of the world. However, in few cases some heavy metals were above permissible limits for European Union which represent the strictest legislations. *C. gallina* examined in the present study was clean especially for contamination with Hg and Cu compared to bivalve studies in other countries as Spain and Turkey.

Spatial distribution of heavy metals in *C. gallina* across the investigated area showed that the clams are in general within safe limits especially in the western and middle parts of the investigated area from Rasheed eastwards. This zone have the maximum biomass of *C. gallina* as confirmed by a simultaneous fishery study (FAO EastMed 2014). This indicates that, *C.* in this zone is safe for human use and might provide promising fisheries for exploitation when toxic risk of the most hazardous heavy metals such Hg, Cu, Cr and Zn are concern. However, the eastern part of the studied area near Burullus must be monitored for Pb contamination and sources. Then, collection of *C. gallina* should be avoided when necessary.

## AQUACULTURE DEVELOPMENT IN EGYPT

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Aquaculture is currently the largest single source of fish supply in Egypt accounting for almost 77 percent of the total fish production of the country in 2014 with over 99 percent produced from privately owned farms. The development and expansion of modern aquaculture began in Egypt two decades ago, with relative declining of capture fishery (344,791 tons in 2014), it becomes obvious that aquaculture is the only viable option for reducing the gap between fish production and consumption in Egypt. Aquaculture is practiced using a variety of systems with varying levels of technology. The majority of farmed fish are either freshwater species or those that can grow in brackish water while marine aquaculture farms are going on now. The majority of fish farms in Egypt can be classified as semi-intensive brackish water pond farms. Intensive aquaculture, in earthen ponds and tanks, is now developing rapidly to counter act the reduction in the total area available for aquaculture activity as well as the scarcity of water in some locations.

The high rate of return on investment in aquaculture has attracted a large number of small to middle level investors. The sector is becoming more sophisticated and diverse and this is also associated with a rapid expansion of the support activities such as feed mills and hatcheries as the number of fish hatcheries has drastically increased reaching over 560 in year 2014 and Wild fry collection stations increased from 69 in 2005 to 72 in year 2014. (GAFRD, 2014). In regard to feed mills, there are more than 16 fish feed manufacturing companies of commercial size while there are larger number of small to middle class sizes.

Historically, the first semi-intensive commercial-size farm in earthen ponds was built by the Government in 1961 using ploy - culture system. By the mid 1980s, annual aquaculture production had jumped from a mere 17 000 tones to 45 000 tones. The average annual production from these semi-intensive systems was in the range of 1.5–2.5 tons per hectare. Afterwards, during 1990s, aquaculture activities steadily developed whereas aquaculture production increased from 539,747 tons in 2005 to 1,137,091 tons in 2014. The production from private fish farms amounted 916,757 tons, followed by 176,266 tons produced from cages and then 33,978 tons from rice fields.

Egyptian aquaculture is characterized by its narrow production basket dominated by tilapia that represented about 67%, followed by carps (17.5%), grey mullet 11% leaving only about 5.0% to other species which includes catfish, marine finfish and shrimp.

## EXTRUDED FEED/FOOD QUALITY MANAGEMENT. RELATIONS BETWEEN TECHNOLOGY AND EXTRUDED FEED/FOOD QUALITY

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Extruded feed/food quality management

Relations between technology and Extruded feed/food quality

Agenda

1. Extruded feed/food quality management
2. Outline of extrusion production flow
3. Definitions of good extruded feed/food quality
4. ANDRITZ machines and technologies for extruded feed/food production

### Extruded feed/food quality management

- One of the keys to successful fish farming lies in correctly adapted feed formulas, high quality ingredients, controlled homogenous content and optimum physical feed pellet properties. Pet food requires “complete and balanced” diets, which means correct nutritional values and palatability.

- In order to keep pace with these increasing demands, industrially produced feed/food is undergoing a continuous quality improvement process. The different types of feed/foods are subject to different demands, the feed/food’s functionality. E.g. on fish farms in terms of floatability or sink ability and the pellets durability to assist mechanical handling without generating of fine.

### Extruded feed/food quality management

- **What is good quality and how to** affect it is the main subject in feed/food production lines. The technical quality in the different areas in the feed/food production line includes fine grinding, conditioning before the extrusion process, extrusion, drying, vacuum coating and pellets cooling etc.

- **Technical quality means for example:** Density, sinking or floating ability, fat, moisture, dimensions (length, diameter), dust etc.

- **Chemical quality can mean many things:** How to protect nutrient agents from damage in the feed/food processing line and in the storage prior to the feeding.

- **Challenges with new raw material:** The traditional feed/food ingredients are limited. How to adapt to new ingredients and raw material and get the most out of them.

- **Increased focus on food safety:** Feed/food ingredients and process traceability.

- **How to ensure high-quality feed** at minimum operating costs

Extruded feed/food quality management

ANDRITZ Feed & Biofuel

**Chemical quality is mostly related to the challenge of optimization of available raw material to match the requirements of each species of animal:**



- Recipe in respect to agreement between the manufactory and “customer” (fish farmer, marketing, veterinarian)
- Raw materiel / recipe in respect to accessibility, prizes
- Crude protein, crude lipids %, digestible protein, digestible energy, moisture, ash, etc.
- Feed micro ingredients: Vitamins, minerals, digest and other.
- In the processing of feed/food, the protection of various ingredients, e.g. in fish feed proteins and amino acids is of high essence. This means management of temperature, time and moisture.

### **Trends and related challenges in the pet food industry**

#### Change in pet food ingredients concepts

- 1) Less grain and traditional starch sources
- 2) Increased inclusion of exotic ingrediens, food by-products and vegetable proteins, e.g chia seeds, various types of fresh meat inclusions,

#### Trends and related challenges in the aquatic feed industry

#### Change in feed ingredients concepts

- 1) Less marine proteins and oils
- 2) Increased inclusion of vegetable proteins and oils and food by-products

### **Feed ingredients relations to quality and drying properties**

#### **What is good technical quality:**

- Durability, density, floating / sinking, avoiding broken pellets and dust , securing uniform and smooth surface of the pellets.
- Texture profile analyses of stress relaxation test
- Weight, dimension length / diameter

### **Quality management**

#### **Grinding Andritz Hammer Mill**

**In extrusion processes, each sub-process impacts the performance of the next step in the process.**

- Finished product quality
- Fine structures
- Reduce fines
- Easier access to carbohydrates via smaller practical

### **Extrusion**

#### **Extrusion cooking parameters influence on the final product**

#### Quality in coating

- Gentle handling
- No oil leaking from pellets
- Dry surface
- No fines
- No breaking pellets
- Uniformity of product

### **Extruded feed technology solutions – we offer...**

## THE EFFECTS OF DIETARY FISH OIL REPLACEMENT BY PLANT OILS ON THE GROWTH, WHOLE BODY AND TISSUE FATTY ACID AND NUTRIENT COMPOSITION AND DIGESTIVE ENZYME ACTIVITIES IN NILE TILAPIA (*Oreochromis niloticus* L.)

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This study aimed at demonstrating the effects of dietary fish oil replacement by plant oils on the growth, whole body and tissue fatty acid and nutrient composition and digestive enzyme activities in Nile tilapia (*Oreochromis niloticus* L.). Five iso-nitrogenous and iso-lipidic diets (34 % CP and 10 % CL on a dry matter basis) were formulated to replace dietary Fish Oil (FO) by sunflower seed oil (SF), Canola Oil (CO), Linseed Oil (LO) and equal combination (BLD;1:1 w:w) of these vegetable oils. A total of 375 fish (32.5±0.5 g) were randomly assigned to 15 190 l rectangular fiberglass tanks and fed 3 % BW/d two times in equal amounts both for morning and afternoon feeding.

Fish tripled its initial weight and no significant difference was observed among growth rates of fish in different dietary treatments. Fish fed the diets containing vegetable oils had lower Hepato Somatic Index compared to that of fish fed FO diet and the difference was statistically significant ( $p<0.05$ ) between fish fed SF and FO diets. Fatty acid compositions analysis of whole body and tissues of muscle, liver and gill showed that the intermediates of n-3 LC HUFA biosynthesis pathway from  $\alpha$ -linolenic acid were more favourably expressed in fish fed SF diet compared to that of fish fed other dietary treatments. It was also evident that EPA was readily oxidized regardless of the dietary treatments whereas DHA was proportionally retained in significantly higher ( $p<0.01$ ) rates in fish fed diets containing vegetable oils compared to that of fish fed FO diet. Dietary treatments did not affect the trypsin activities in fish significantly but slight increases were recorded with fish oil inclusion in diets.  $\alpha$ -amylase activity of fish fed CO diet was found to be significantly higher ( $p<0.05$ ) than that of fish fed other dietary treatments. Furthermore, lipase activities in fish fed SF and CO diets were also measured significantly higher ( $p<0.01$ ) than that of fish fed other diets indicating lipolytic activity in Nile tilapia was stimulated by medium chain fatty acids specifically linoleic and oleic acids.

This study demonstrated that up to 80 % of total fish oil in Nile tilapia diets could be replaced by either solely or in equal combinations of sun flower, canola and linseed oils without compromising growth rates, whole body and tissue fatty acid compositions and nutrient absorbent efficiencies. Further research targeting total fish oil replacement by these vegetable oils with special consideration to fatty acid metabolism, organ histology and immune response would be highly useful in this species

## EFFECT OF LIGHT LIMITATION ON THE WATER QUALITY, BACTERIAL COUNTS, AND PERFORMANCE OF *Litopenaeus vannamei* POSTLARVAE REARED WITH BIOFLOC AT LOW SALINITY AND ZERO-WATER EXCHANGE

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The aim of this study was to evaluate the effect of light limitation on the water quality, bacterial counts, and performance of *Litopenaeus vannamei* postlarvae reared with biofloc at low salinity and zero-water exchange. Two treatments were designed:  $T_1$  = Culture with natural sunlight and  $T_2$  = Culture in darkness. After 28 days, in both treatments, the final weight of shrimp was over 0.6 g with a specific growth rate (SGR) over 7.4%/d, and a survival rate over 70%. In both treatments, *Vibrio* sp. concentration presented low values (culture with natural sunlight = 0.1 to  $9.9 \times 10^2$  CFU/mL, culture in darkness = 0.4 to  $11.7 \times 10^2$  CFU/mL) and *Bacillus* sp. had high values (culture with natural sunlight = 0.7 to  $66.0 \times 10^4$  CFU/mL, culture in darkness = 0.7 to  $65.8 \times 10^4$  CFU/mL). All water quality parameters remained within the ranges suitable for shrimp culture, except for alkalinity during the first stage of the study. Although in some sampling periods, some significant differences were found in bacterial counts and water quality parameters, shrimp productive performance under culture with biofloc at low salinity ( $\approx 9$  ‰) and zero-water exchange was not significantly affected by light limitation.

## EFFECT OF WATER EXCHANGE AND STOCKING DENSITY IN A BIOFLOC-BASED SYSTEM ON SALINITY STRESS TOLERANCE AND PERFORMANCE OF *Litopenaeus vannamei* POSTLARVAE

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The effect of water exchange and stocking density was evaluated on the salinity stress tolerance (by low and high salinity) and performance of *Litopenaeus vannamei* postlarvae reared in a biofloc-based system at nursery level. A bioassay was conducted with four treatments in triplicate:  $T_1$  = 8250 orgs/m<sup>3</sup> without water exchange,  $T_2$  = 16,500 orgs/m<sup>3</sup> without water exchange,  $T_3$  = 8250 orgs/m<sup>3</sup> with 50% water exchange per week, and  $T_4$  = 16,500 orgs/m<sup>3</sup> with 50% water exchange per week. At 0, 15, 30, and 45 days of culture, a group of shrimp from each treatment was exposed to acute stress by low (27 to 2 ‰) and high salinity (27 to 60 ‰). After 49 days, the water exchange rate and water exchange rate  $\times$  stocking density interaction affected shrimp survival, but shrimp growth was affected only by stocking density. The mean weight of shrimp in the treatments with the lowest stocking density was higher than that with the highest stocking density, independently of the water exchange rate. In the salinity stress tests, shrimp survival was only affected by the interaction between the salinity stress tests and treatments ( $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$ ). Generally, shrimp showed a greater tolerance when were subjected to low salinity (27 to 2 ‰). In the culture with biofloc at nursery level, apparently, shrimp from the treatments with water exchange improved their tolerance to stressful events caused by changes in salinity, but this response was not related to growth and survival.

## FEED STIMULANTS FROM GREEN ALGA *Ulva* FOR SEA URCHIN *Tripneustes gratilla*

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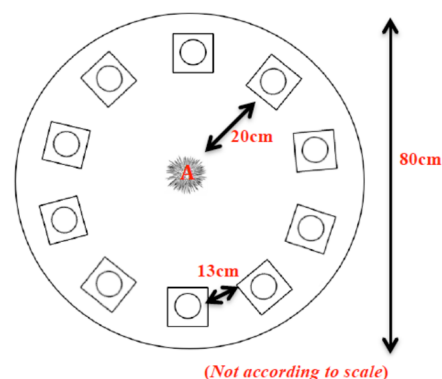
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Secondary metabolites play a fundamental role in mediating ecological interactions for marine plants. Much evidence of this chemical mediation comes from plant/herbivore interactions, where it is clear that some metabolites act as allelochemicals. However, other ecological interactions such as feed stimulants or attractants are less well known. Local aquacultured sea lettuce (*U. armoricana*) is known to elicit phagostimulatory responses in the sea urchin *T. gratilla*, with several studies showing a feeding preference for this alga. The present study investigated the feeding preference of *T. gratilla* for several *Ulva* fractions with the prospect to identify chemical compounds that may act as a feed stimulant or attractant.

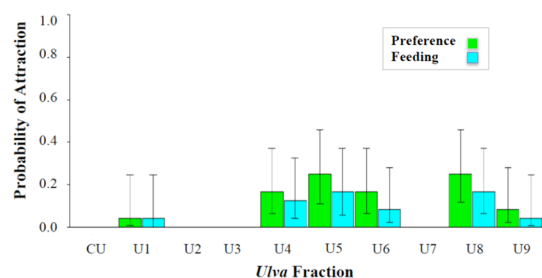
The touch and feed preferences of *T. gratilla* were investigated for nine *Ulva* fractions (obtained from step gradient SI-gel column of crude extract), in a circular tank for 75 minutes using a modified version of the 'Avicel' plating technique from Sakata *et al.* (1984; Figure 1). Overall, 24 experiments were carried out on urchins ( $\delta$  3.8–5.4 cm), with bioassay plates randomized for each experiment.

Touch preference: 23 out of 24 urchins were responsive to *Ulva* fractions. The highest number of observations was observed for fractions U5 and U8 followed by U4, U6 and U9 ( $p < 0.001$ ). Feed preference: 14 out of 24 urchins showed a feeding preference. Fraction U5 was mostly preferred followed by U4, U8 and U6 ( $p < 0.05$ ). Binomial GLM showed that fraction U5 and U8 had the highest probability to attract urchins and the highest probability of urchins feeding on them (Figure 2).

NMR spectroscopy of fractions U4, U5 and U6 revealed the presence of lipids such as methyl palmitate (isolated from U4 and U5) whereas U8 and U9 consisted mostly of complex glycolipids. Results show clear evidence of the presence of compounds in *Ulva* that may act as a feed stimulant or attractant for the sea urchin *T. gratilla*.



**Figure 1:** Experimental set-up.



**Figure 2:** Probability of urchins attracted to specific *Ulva* fractions.

## FISHERY WASTES MANAGEMENT: THE AQUAFEED OPTION

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Large quantities of low-value fishes are landed from marine and freshwaters in Nigeria in short periods and often glut the market. Consequently, much of these remain unsold and spoil due to poor handling and processing. Fishery wastes are thus generated and abound in the coastal regions, where they create disposal problems. The current disposal practices include burial, municipal garbage disposal and dumping in fields or streams. An alternative disposal method is the conversion into fish meal or fish silage, and used as aquaculture feed supplements. The objective of the study was to provide information to facilitate low-cost production of farm-made dry meal or fermented silage from fishery wastes by biological fermentation for potential use in aquafeeds

Fishery wastes products (whole fish, heads, and viscera) were derived from low oil fishes, obtained from commercial fish processing operators in coastal regions of southern Nigeria; and separated into batches A. and B. Batch A was converted into fishery wastes meal (FWM) using the conventional cottage dry fish meal industry practice in Nigeria. Batch B was converted into fishery wastes silage (FWS) using lactic acid fermentation methods. Fishery wastes meal (FWM) and fishery wastes silage (FWS) (fermented) were evaluated as substitutes for herring meal (HFM) in a reference diet. Growth response, feed utilization and carcass composition of catfish fed with the diets were similar. Fishery wastes could be economically utilized to produce acceptable high-protein feedstuffs (meal, silage) for aquafeeds. Fermented fish silage has been used as feed supplement for various aquaculture fishes and they possess good nutritional quality, and the biological value of its protein was comparable with that of fish meal protein. The production of meal and silage appears an attractive way of utilizing fishery wastes, and will reduce the foreign currency drain presently involved in importing herring fish meal. However, aquafeed production should not overshadow the benefit of direct human consumption of low value fishes in the fight against malnutrition in Nigeria.

Table 1: Growth, feed utilization, carcass composition (g/100g) (%) of catfish fed with experimental diets.

|                              | HFM               | FWM                | FWS               |
|------------------------------|-------------------|--------------------|-------------------|
| Initial wt. (g)              | 12.1              | 12.1               | 12.1              |
| Final wt. (g)                | 62.7              | 61.2               | 58.6              |
| Wt. gain (g)                 | 50.6 <sup>a</sup> | 49.1 <sup>ab</sup> | 46.5 <sup>b</sup> |
| ADG (g/day)                  | 0.72 <sup>a</sup> | 0.70 <sup>a</sup>  | 0.67 <sup>b</sup> |
| SGR                          | 2.35              | 2.33               | 2.19              |
| FCR                          | 1.42              | 1.43               | 1.50              |
| PER                          | 1.81              | 1.80               | 1.78              |
| Survival (%)                 | 100               | 100                | 100               |
| Carcass composition (g/100g) |                   |                    |                   |
| Moisture                     | 78.6              | 75.6               | 75.5              |
| Protein                      | 13.9              | 16.2               | 16.4              |
| Lipid                        | 5.1               | 6.2                | 6.1               |
| Ash                          | 2.7               | 2.5 <sup>b</sup>   | 2.7 <sup>a</sup>  |
| HSI                          | 1.5               | 2.0                | 2.1               |

Mean values in a row with dissimilar superscripts are different (P<0.05).

Table 2: Nutrient digestibility (%) of catfish fed with experimental diets.

|                            | HFM               | FWM               | FWS               |
|----------------------------|-------------------|-------------------|-------------------|
| Nutrient digestibility (%) |                   |                   |                   |
| Dry matter                 | 88.0              | 84.7              | 85.4              |
| Protein                    | 82.5              | 80.2              | 81.1              |
| Lipid                      | 71.6              | 72.0              | 72.6              |
| Energy                     | 79.2 <sup>a</sup> | 75.8 <sup>a</sup> | 70.8 <sup>b</sup> |

h dissimilar superscripts are different (P<0.05).

## ANTIFERTILITY ACTIVITY OF NONI (*Morinda lucida*) LEAF MEAL ON NILE TILAPIA (*Oreochromis niloticus*)

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Tilapias are tropical aquaculture fish species and are yet to reach their full potential because of the problems of precocious maturity and uncontrolled reproduction, which often result in the overpopulation of production ponds with young or stunted fish. There is need to control unwanted/undesirable tilapia recruitment in ponds using natural reproductive inhibitory agents in ethnobotanicals (plants) because they are less expensive and constitutes appropriate technology in developing countries. *Morinda lucida* leaf meal was added to a basal diet (350g crude protein, 18.5MJ GE/kg diet) and fed at low dose (5 g/kg/day) or high dose (10 g/kg/day) to male or female *Oreochromis niloticus* for 60 days; to evaluate effects on reproduction traits and gonad histology.

There were no differences ( $P>0.05$ ) in growth and feed conversion indices. Permanent sterility was induced in tilapias in the high dose treatment, while sterility in the low dose treatment was reversible, with no significant loss in fish weight ( $P>0.05$ ). Both dose treatments decreased sperm motility, testis mass and sperm count, egg diameter; and inhibited gonadal development. Gonadal histology showed swollen testes nuclei in low dose treatment and disintegrated cells in high dose treatment; and varying degrees of disintegration of follicle cells and atrophy of ovarian nuclei in high dose treatment. The severity of cell destruction and vacuolation showed that antifertility activity was dose dependent. Reproduction traits, gonado-somatic index (GSI) as well as gonadal development and histology in *O. niloticus* fed high doses revealed that *Morinda* leaves were effective as antifertility agents, representing an appropriate technology that is easily adopted by resource-limited African fish farmers, as effective anti-fertility agents for farm-raised tilapias and to control prolific breeding which cause stunted tilapia.

Table 1. Growth and feed conversion indices of *O. niloticus* fed with *Morinda* leaf meal diets.

|                       | Control | □low dose | □high dose | □low dose | □high dose |
|-----------------------|---------|-----------|------------|-----------|------------|
| Final wt.             | 58.6a   | 58.5a     | 58.2ab     | 57.7bc    | 56.9c      |
| Wt. gain (g)          | 18.4a   | 18.2a     | 17.9b      | 18.0b     | 19.7c      |
| % wt. gain            | 45.7a   | 45.3a     | 44.7b      | 43.5bc    | 41.4c      |
| Specific Growth rate  | 30.6    | 30.4      | 30.1       | 30.1      | 29.8       |
| Feed conversion ratio | 2.2     | 2.2       | 2.2        | 2.2       | 2.3        |

Initial wt. - 40.2g; Means in a row with different letters are significantly different ( $P<0.05$ ).

Table 2: Reproduction traits and histological description of *O. niloticus*

| Sex | Treatments | GSI (%) | Histological description   |
|-----|------------|---------|--|
| □   | Low dose   | 1.13    | slight alterations in testis architecture                        |
|     | High dose  | 1.27    | cystic seminiferous tubules and tissue atrophy                   |
| □   | Low dose   | 1.33    | slight alterations in ovarian architecture                       |
|     | High dose  | 1.48    | severe tissue atrophy, ovarian cells disintegration and necrosis |



## EFFECTS OF REPLACEMENT OF TOMATO WASTE MEAL BY MANGO KERNEL MEAL ON GROWTH PERFORMANCE, SURVIVAL AND CARCASS COMPOSITION OF TILAPIA *Oreochromis niloticus*

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The objectives of this study were to substitute the tomato waste meal with the mango kernel meal in the diet of *Oreochromis niloticus* fry in order to increase the sources of nutrients, also to determine the best rate of incorporation. Five (5) diets were formulated with inclusion rates 0; 2.5; 5; 7.5 and 10% of mango kernel meal and then tested on tilapia fry of an average initial weight of 0.34 g for sixty (60) days. The stocking density was 10 fish per tank in an isolated system consisting of 10 plastic tanks of 50 liters.

At the end of the experiment, the best absolute average weight gain (2.05 g); relative mean weight gain (603%) and specific growth rate (3.25%) were recorded in the fry fed with the diet containing 2.5% mango kernel meal incorporation. These results are not statistically different from those obtained with fish fed diets containing 0 and 5% inclusion of mango kernel meal. Fish fed diets containing 7.5 and 10% mango kernel meal presented the lowest results. In terms of feed efficiency, fish fed the diet 2.5% has the best feed conversion rate (1.54). This FCR is not statistically different from that of the Fish fed diet 5% (1.77). The diets containing 0; 7.5 and 10% have the lowest FCR. The fish fed diet containing 2.5% mango kernel meal also has a better protein efficiency ratio (1.91). This result is not statistically different from that of other diets. The fish fed the diets containing 2.5; 5 and 7.5% of mango kernel meal have the best survival rate (95%), while the control diet 0% has the lowest survival rate (75%). With the increase in the incorporation rate of mango kernel meal, proteins tend to increase, lipids and dry matter decrease in the flesh of processed fish.

In conclusion, mango kernel meal can substitute up to 5% of tomato waste meal in *Oreochromis niloticus* feed without affecting growth performance, feed efficiency ratio and survival. However the best performances are recorded with the diet containing 2.5% of mango kernel.

**Table I : Composition of diets**

|                          | 0%  | 2.5% | 5%  | 7.5% | 10% |
|--------------------------|-----|------|-----|------|-----|
| <b>Fish meal</b>         | 150 | 150  | 150 | 150  | 150 |
| <b>Peanut meal</b>       | 430 | 430  | 430 | 430  | 430 |
| <b>Cassava meal</b>      | 20  | 20   | 20  | 20   | 20  |
| <b>Tomato waste</b>      | 350 | 341  | 333 | 324  | 315 |
| <b>Mango kernel meal</b> | 0   | 9    | 18  | 27   | 35  |
| <b>Fish oil</b>          | 50  | 50   | 50  | 50   | 50  |

**Table II: Growth parameters**

|                 | 0%     | 2.5%   | 5%     | 7.5%   | 10%    |
|-----------------|--------|--------|--------|--------|--------|
| <b>IMW (g)</b>  | 0.34   | 0.34   | 0.34   | 0.34   | 0.34   |
| <b>FMW (g)</b>  | 2.24   | 2.39   | 2.18   | 1.58   | 1.69   |
| <b>AMWG (g)</b> | 1.9    | 2.05   | 1.84   | 1.24   | 1.35   |
| <b>RMWG (%)</b> | 560.29 | 602.94 | 542.65 | 364.71 | 398.53 |
| <b>FCR</b>      | 2.20   | 1.54   | 1.77   | 2.51   | 2.32   |
| <b>SGR</b>      | 3.14   | 3.25   | 3.10   | 2.56   | 2.67   |
| <b>PER</b>      | 1.34   | 1.91   | 1.66   | 1.17   | 1.27   |
| <b>S %</b>      | 75     | 95     | 95     | 95     | 90     |

**Table III: Carcass composition**

|             | Initial fish | 0%    | 2.5%  | 5%    | 7.5%  | 10%   |
|-------------|--------------|-------|-------|-------|-------|-------|
| <b>DM %</b> | 96.33        | 96.71 | 95.86 | 94.12 | 93.87 | 93.92 |
| <b>CP %</b> | 49.63        | 47.71 | 44.89 | 51.4  | 59.82 | 60.34 |
| <b>CL %</b> | 41.51        | 41.08 | 38.47 | 36.12 | 25.1  | 25.86 |



## GROWTH PROGRESSION OF HATCHERY SPAWNED SILVER CATFISH (*Chrysichthys nigrodigitatus*) LARVAE IN A CONTROLLED INDOOR ENVIRONMENT

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A study on the growth trend of fry of the silver catfish, *Chrysichthys nigrodigitatus* reared in 5.25L capacity plastic tanks in a wet laboratory was conducted. Two hundred and fifteen fry of *C. nigrodigitatus* with an initial mean weight of  $0.02 \pm 0.00$ g were stocked in triplicates and the fry were fed eight times daily with 0.2-0.3mm 56% CP extruded starter diet (Coppens©) for four weeks. Thereafter, six times a day for another four weeks with 0.3-0.5mm Coppens© feed and at the last phase; thrice daily for two months with 0.5-0.8mm Coppens© feed coupled with twice daily water exchange. The final mean weight was  $7.67 \pm 6.25$ g at the end of the 16 weeks of study. The growth parameters were taken using growth indices that includes weight gain (WG), percentage weight gain (PWG), percentage mean weight gain (PMWG), specific growth rate (SGR), food conversion ratio (FCR), gross food conversion ratio (GFCR), protein intake (PI) and nitrogen metabolism (NM). The mean daily weight gained was 9 mg/day, the weight gain was  $7.67 \pm 6.25$ , the percentage weight gain was  $64.08 \pm 55.34$ , the specific growth rate was  $3.12 \pm 1.82$ , the food conversion ratio was  $2.64 \pm 1.42$ , the gross conversion ratio was  $48.63 \pm 26.54$ , protein intake  $228.36 \pm 86.93$  and nitrogen metabolism was  $236.56 \pm 192.13$  respectively. The results of this study indicated that fry of *C. nigrodigitatus* readily accept the commercial diet; Coppens and could be used to successfully rear the fry of *Chrysichthys nigrodigitatus* thus providing alternative to catfish in the aquaculture industry in Nigeria and West Africa where it is endemic.

## **A REDUCED REPRESENTATION OF THE DNA METHYLOME IN FAST MUSCLE OF NILE TILAPIA *Oreochromis niloticus***

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Nile tilapia (*Oreochromis niloticus*) and other tilapiine cichlids are among the most important aquaculture fish species worldwide, with a long history of farming and worldwide distribution. The growing interest in this species in the last decades has been supported by extensive studies focusing on enhanced growth performance, which is one of the critical parameters of fish farm productivity. Today, aquaculture tilapia's growth performance has been dramatically increased by genetic improvements (selective breeding) and environmental conditions, i.e. specific diet or rearing density. However, although genotype selection is in the process of being optimized, little is known about the epigenetic mechanisms involved in growth regulation. In order to get insights into epigenetic mechanisms regulating growth phenotype in tilapia, we used reduced representation bisulfite sequencing (RRBS) to compare the fast muscle methylomes of slow and fast growing fish from the same cohort. To the best of our knowledge, this is the first report employing RRBS to characterize DNA methylation of the reduced genome at a single base level in Nile tilapia. The RRBS analysis showed differential methylation of CpG islands situated close to the promoters of functionally relevant genes. We discuss the potential implication of these results in growth performance of farmed Nile tilapia and sustainability of the aquaculture industry.

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement n° 683210).

## TILAPIA FARMING VIA RYDAWI'S FARMINABOX - FROM POND TO RECIRCULATION AQUACULTURE SYSTEM FOR AFRICA

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How do we address the current shortage fish for consumption on the African continent? Market trends show that expectation of the consumption of fish will be doubled over the next 50 years. The fact is that there are a low number of commercial fish production operations on the continent, and those that are established are favouring pond and cage farming systems.

Pioneers in this industry have to consider a number of factors when creating new innovative ways to pique the interest of potential developers, farmers and investors, as well as increasing production. These factors include access to good fingerling stock, low cost of a production system, and a system that is robust, flexible, scalable, easily moved and user friendly.

As the table shows the increase in production of a Tilapia farm is higher if the fish are farmed in a tank system, compared to pond farming:

Systems that are built around Recirculating Aquaculture System (RAS) design principles offers a unique and simple way for anyone to enter the world of fish farming with the lowest cost of entry, lowest running costs and lowest technical requirements – and most importantly a higher production rate. The RAS closed-containment aquaculture systems represent a quantum leap forward, both as a lucrative and low-risk business venture as well as the most sustainable and efficient form of aquaculture today, with efficiencies more than 10 times conventional fish farms. Next-generation systems recirculate up to 99 percent of their effluent, have no discharge, use no chemicals or antibiotics, and can be sited close to market, resulting in a fresher product and dramatically lower transportation costs.

| Small Scale Tilapia Production In Africa |                        |       |        |            |
|--|------------------------|-------|--------|------------|
| Traditional Pond Farming                 |                        |       | Versus | FarmInABox |
| Parameter                                | Unit                   | Value | Vs     | Value      |
| Volume                                   | m <sup>3</sup>         | 120   |        | 11         |
| Water Use                                | m <sup>3</sup> / Annum | 240   |        | 42         |
| Manpower                                 | People                 | 2     |        | 1          |
| Stocking                                 | # of Fish/Cycle        | 600   |        | 285        |
| Harvest mass                             | Kgs/cycle              | 120   |        | 66         |
| Production Cycle                         | Days                   | 270   |        | 150        |
| Production per year                      | Kgs/year               | 162   |        | 787        |
| Production per year                      | # of Fish/Year         | 811   |        | 3,420      |

## INVESTIGATING SEASONAL VARIABILITY IN THE CONTAMINANT COMPOSITION OF THE COMMERCIALY CULTURED NATIVE BLACK MUSSEL (*Choromytilus meridionalis*) AND INVASIVE MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*) IN SALDANHA BAY, SOUTH AFRICA

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Mussel aquaculture is the second largest marine aquaculture subsector in South Africa, producing approximately 860 tons per annum, and providing much-needed jobs in poorer coastal fishing communities. However, the dynamic and rough oceans along the South African coastline have restricted mussel mariculture to sheltered bays of which there are few. Saldanha Bay, located on the west coast, is one such suitably sheltered bay and is currently the location of all national mussel aquaculture production.

There are 7 aquaculture operators active in Saldanha Bay, of which 3 are dedicated mussel farms. However, pollution within the bay is of increasing concern due to a growing human population, insufficient waste water treatment, high volume of shipping traffic and dredging, as well as the presence and planned expansion of an extensive iron ore terminal. These pollution concerns are further exacerbated by the fact that the quay and iron ore jetty, which jut out into the Bay, have caused decreased rates of water replacement.

Bivalves are filter feeders and known bioaccumulators of heavy metals, pesticides and Persistent Organic Pollutants (POPs) present in their environment, which can have considerable health implications, such as cancers and infertility, when consumed by humans, due to biomagnification along the food chain.

This study has therefore assessed the levels of heavy metals, pesticides and POPs in commonly consumed and farmed mussel species (*Choromytilus meridionalis* and *Mytilus galloprovincialis*) from Saldanha Bay over a two-year period and identified changes due to seasonal fluctuations in precipitation. The pollutants investigated included twenty-six heavy metals such as mercury and lead, a wide range of pesticides including Dichlorodiphenyltrichloroethane (DDT), which has been found to persist in the environment despite its use being strictly controlled in South Africa, and several POP's such as polychlorinated biphenyls which are thought to be introduced into the environment through Saldanha's wastewater.

These results can be used by stakeholders such as mussel farmers and residents who collect mussels recreationally, as well as consumers and food producers to determine baseline pollution data, identify optimal harvest times and local industrial effects (iron ore-present and future) as well as potential health implications. The study could also potentially be used to encourage investment in the mussel sector, which could in turn help uplift poorer communities in Saldanha, as investment in bivalve aquaculture has been found to have the highest employment per unit of any aquaculture sector in South Africa.

## INDIGENOUS FISH AQUACULTURE AS A MEANS FOR THE SUSTAINABLE DEVELOPMENT OF RURAL COMMUNITIES REMAINING IN NATURE RESERVES OF BRAZIL

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Aquaculture has been proposed as a tool for social development, human nutrition, and health. Lambari aquaculture in Brazil has been performed by many small farmers in rural areas with little access to investment and infrastructure. As an indigenous, low-trophic level fish with high nutritional value, this species group has a good potential to be developed in sustainable way and promote community health and welfare. To estimate such prospects, we performed a preliminary assessment of environmental sustainability of lambari (*Deuterodon iguape*) aquaculture at a single, semi-intensive farm. The analysis included three aspects: use of natural resources, efficiency of use of natural resources, and release of pollutants to the environment.

The study was conducted on Guanhã City Hall Aquaculture Farm (24°12'26.12" S, 47°2'48.24" W). This farm is located in a rural community bounded by the Sea Mountains State Park, an Atlantic Rainforest protected area adjacent to the ocean in the southeast of Brazil. Today about 800 poor families are allowed to live in this area as long as their livelihoods are in accordance with the park's aims.

The current aquaculture system performed poorly in all measures of sustainability examined. The lambari aquaculture system had a high consumption of natural resources, and low resource use efficiencies. The main issues were related to water and nutrient usages. Lambari aquaculture consumed about 3X more water, phosphorous and nitrogen per ton of fish produced than other comparable semi-intensive aquaculture systems in Asia and Africa. Less than 40% of applied nutrients were recovered in fish biomass. Lambari productivity was also very low when compared to those systems and other aquaculture systems in Brazil. The reasons for the poor performance of the system were inappropriate pond management and diets.

Nevertheless, lambari aquaculture offers no risk of impact to local biodiversity, no hormone/pesticide releases, and can be much less harmful to the environment compared to exotic fish culture or other intensive animal protein production systems. Moreover, an adequate management regime can be devised to improve the system's efficiency.

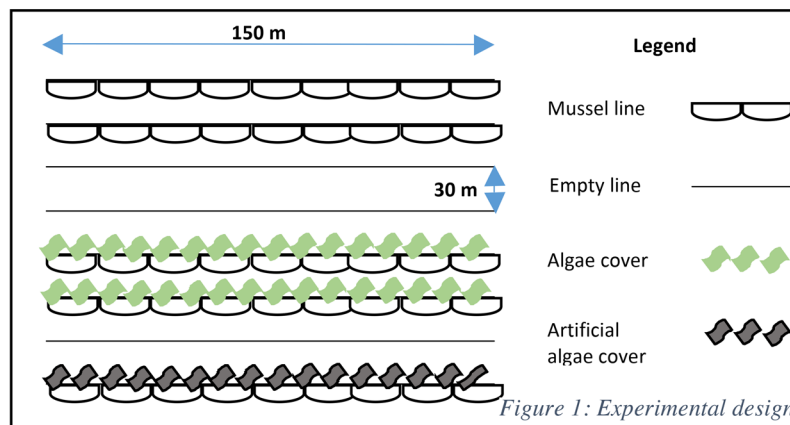
With additional participatory, applied science, the aquaculture of indigenous, low trophic level, highly nutritious fish such as the lambari can be an important tool for sustainable food production and alternative development of rural populations that could fit well within the social-ecological context of protected areas in Brazil.

# CO-CULTURE OF BLUE MUSSEL *Mytilus edulis* AND SUGAR KELP *Saccharina latissima*: EXPLORING THE POTENTIAL EFFECT OF SEAWEEDS IN DETERRING THE EFFECT OF DUCK PREDATION ON MUSSELS, CASCAPEDIA BAY (QC, CANADA)

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In Europe and Canada the economic losses in blue mussels (*Mytilus edulis*) farms due to duck predation represent a major problem. In this project, an alternative approach will be presented that aims to reduce duck predation in mussel farms as traditional techniques are generally neither effective nor cost efficient. The traditional methods are generally either focusing on protecting mussels by isolating them (net, protective socks and cages), using passive deterrent (mannequins, balloon, mirrors, corpses, etc.) or active repelling techniques (sound, light, chase, lethal force, etc.). These techniques are often expensive, potentially stressful to duck populations, only effective for a short period of time and do not take into consideration drifting ice. When mixed together, randomly used and interchanged over time, they are believed to be more effective by reducing ducks potential of habituation but also reducing considerably the profit margin. To solve this problem, the presented project intends to introduce brown algae (*Saccharina latissima*) in co-culture over a mussel floating line, to visually shield the mussels. We hypothesise that by hiding the mussels from the ducks vision field, it will protect the production without imposing further stress on the ducks. Additionally, it is expected that the brown algae and blue mussel could benefit by the spatial proximity in terms of production, consumption and excretion. On the farm production, such design allowing a circular economy where the repellent is also a product could ease the losses recovery while developing a polyculture model for the farmers. This experiment, beginning in September 2016, will be harvested between April and May 2017. Analysis will include the observation of resulting growth (biomass \ meter), survival rate (density \ meter) and overall mussel quality (Body condition Index) of the floating lines and compare them to a neighbouring line without algae cover and another one with artificial algae cover made of polypropylene sheets.



## ACCUMULATION OF PARALYTIC SHELLFISH POISONING TOXIN IN THE ABALONE *Haliotis midae* AND POTENTIAL VECTORS

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Paralytic shellfish poisoning (PSP) is a foodborne illness associated with the consumption of seafood products contaminated with the neurotoxins known collectively as saxitoxins (STXs). Much is known about the toxin sources, primarily certain dinoflagellate species, and there is extensive information on toxin transfer to filter-feeding molluscan bivalves, known as the traditional vectors of these toxins. However considerable uncertainty exists as to the source and transfer of these toxins in non-traditional vectors such as abalone. Saxitoxins in the South African abalone *Haliotis midae* were first detected in 1999 and linked to the presence of the dinoflagellate *Alexandrium catenella*.

Our study aimed to reassess the presence and seasonality of saxitoxins in farmed and wild abalone on the South African coast, to further investigate the anatomical distribution of toxins and to establish rates of depuration of toxins from abalone. Results show toxins to be more prevalent in both farmed and wild abalone to the west of Cape Point with no indication of seasonal variability. Toxins were found predominantly in the epipodial fringe and to a lesser extent in the muscle and viscera. Abalone size classes showed no significant difference in toxin content.

Depuration experiments run over 6 months showed significant fluctuations in toxin concentrations without significant depuration. These fluctuations in the absence of *Alexandrium catenella* indicate an alternate source of toxin. Future research will investigate cellular localization of the toxins, and bacteria as a possible source of toxins in abalone.

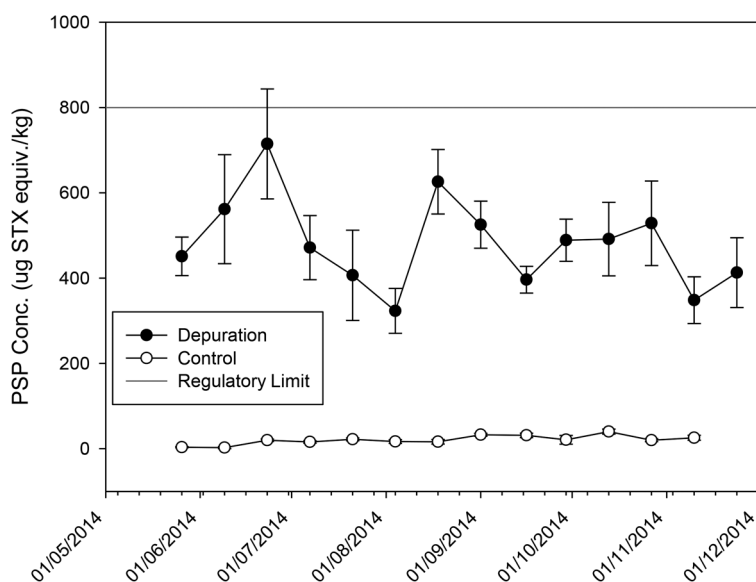


Figure 1: Depuration of the abalone *Haliotis midae* under controlled conditions



## DIVERSITY IN PLOIDY LEVEL OF GYNOGENETIC PROGENY OF STERLET *Acipenser ruthenus* L.

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The gynogenetic development was induced in sterlet *Acipenser ruthenus* L. by activation of eggs obtained from the albino females with UV-irradiated spermatozoa from the wild-colored males followed by the heat shock applied to suppress the first mitotic division in the haploid zygotes. All obtained progeny possessed recessive albino phenotype. Moreover, the molecular verification based on three microsatellite DNA markers confirmed the only maternal inheritance in the albino progeny. In the gynogenetic experimental groups of fish (subjected to the heat shock) the aneuploid, haploid, diploid, triploid, tetraploid and mosaic specimens were identified. Additionally, some individuals from the haploid experimental variants (that were not exposed for the temperature shock) showed diploid chromosome number. Diversity in the ploidy level observed in the gynogenetic sterlets may be the consequence of the spontaneous polyploidization that occurred in the gynogenetic zygotes. Moreover, indicated eggs with varied stages of development and the asynchronous cell cleavages may have resulted in the chromosomal disturbances observed in the gynogenetic sterlets here.

**DEVELOPMENT OF SPERM CRYOPRESERVATION METHODS FOR FOUR SOUTH AFRICAN MARINE FINFISH SPECIES *Argyrosomus japonicus* AND *Pomadysis commersonni* AND *Liza richardsonii* AND *Lithognathus aureti***

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Fish ringer, ASP (artificial seminal plasma), as well as a modified combination of ASP and HBSS (Hanks balanced salt solution) were respectively used as cryo –electrolytes (diluent) and selectively combined with either DMSO or EG (ethylene glycol) in a respective ratio of 9:1 and 9.5:1. The cryosolution-milt mix (1.5ml) was incubated at 4 C° for 2 hours at a dilution ratio of 3:1. Cryovials were then exposed to nitrogen vapor at a height of 3.5cm above liquid nitrogen in closed cryocontainer. A cooling time of 5min was allowed before vials were plunged into liquid nitrogen. Thawing of milt (after 2 weeks) was done in a 50°C water bath for 70 seconds with cryovials in horizontal position to observe thawing completion when mix starts to flow. Post thaw sperm motility was successfully measured for all tested species but with variable success (30-60%) pending the diluent or cryoprotectant used.

*Pomadysis commersonni*



*Argyrosomus japonicus*



*Liza richardsonii*



*Lithognathus aureti*



## BASELINE SOCIO-ECONOMIC STUDY OF SUSTAINABLE INTEGRATED POND BASED AQUACULTURE WITH RICE AND POULTRY PRODUCTION, NIGERIA

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Integrated aquaculture production with livestock have achieved major breakthrough in Asia. Despite the several benefit associated with the production system, not much has been achieved in Nigeria. A baseline study was carried out to assess the perception, levels of awareness and adoption of fish farmers to integrated pond based aquaculture with rice and poultry production. The study area was the wet lands located in the North Central (NC) and South West (SW) agro-ecological zones. A three stage stratified sampling method was used to select fish farmers from agro-ecological zones, states, and Agricultural Development Programme zones. Structured questionnaires were administered to 100 and 200 registered fish farmers from NC and SW respectively. Descriptive statistics; chi-square and linear regression were used for the analysis.

Majority of fish farmers were male (82.2%), attained post secondary education (60.5 %), and had fish farming as their primary occupation (35.9%). More of earthen fish ponds are used for culturing fish compared to concrete tanks. Streams and rivers are the main source of water for fish culture in earthen pond, while most aquaculture production was rain fed, operating during the rainy season only. Due to inability to keep accurate data, 28.6% fish farmers had no data on fish yield and could not declare profit at the end of the production year. A proportion of 42.4% were able to generate annual profit of more than ₦ 300, 000. The most preferred fish species for culture by the fish farmer was *Clarias species*, the African catfish. Sales at farm gate (51.1%) was the main form of disposing the cultured fish. Value addition was by smoking (28.6%). Half of the fish farmers (50.4%) favoured integrated pond based aquaculture with rice and poultry. Only 1.8% adopted rice and 6.5% for poultry, but no fish farmer adopted rice and poultry integrated aquaculture production. Statistical analysis revealed that there was significant difference between the perception of fish farmers on integrated fishing farming within the NC and SW agro-ecological zones ( $P < 0.05$ ). Ecological zone and perception level ( $P < 0.01$ ), household size and willingness to adopt integrated aquaculture ( $P < 0.05$ ) significantly influenced perception of fish farmers to integrated aquaculture.

Many fish farmers were not aware of integrated fish farming. Fish farmers in the NC are more likely to practice integrated fish farming with rice and poultry compared to the SW. Market outlet through whole sellers and middle men are major challenge because few fish farmers were involved in value addition. Though profitable the venture may appear, fish farmers have other challenges that must be addressed. More awareness about the benefits of integrated pond based aquaculture with rice and poultry to fish farmers must be created through extension bulletins in English Language, Yoruba, Hausa and Ibo.

## **USE OF INFORMATION AND COMMUNICATION TECHNOLOGY IN EXTENSION AND ADVISORY SERVICES AMONG FISHERFOLKS AND FISH FARMERS IN ONDO STATE, NIGERIA**

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Internet Communication Technology (ICT) can be used more effectively in fisheries extension and advisory services to pass across information to rural communities, thereby covering more distance and reaching more people. Unfortunately, ICT has not been fully utilized for extension services. The study was therefore aimed to investigate the socioeconomic characteristics of fisher folks, fish farmers, extension personnel, and type of ICT for fisheries extension advisory services in Ondo State, Nigeria.

Two stage stratified sampling method was used to randomly select 60 fisher folks, 50 fish farmers and 40 extension personnel from four zones in Ondo State Agricultural Development Programme (OSADEP). Qualitative data from the Key Informant Interviews and quantitative data from structured questionnaire administered to 150 respondents were collected for the study. Descriptive statistics and inferential statistics were used to analyse the data.

Results show that majority of the respondents were male: fisher folks, 74.58% fish farmers, 80.85%, and extension personnel, 69.23%. Dominant age range was between 41-50 years of age. Majority of the extension personnel (94.87%), fish farmers (89.36%) and fisher folks (88.14%) were married. The extension personnel had varied educational status with (57.28%) having tertiary certificate. Average monthly income of extension personnel was ₦100,000 (30.77%). Extension personnel experience was between 11-15 years (43.59%) in the four zones (OSADEP). Both fisher folks and fish farmers had less than 5 years as (37.29%) and (55.32%) respectively. The mean number of ICT used by fisher folks was 3.69 fish farmers 4.79 and extension personnel was 4.25. Results of chi-square analysis showed that there was significant relationship ( $P < 0.05$ ) in the number of ICT usage between gender and membership of cooperative society for fisher folks; only education was significant for fish farmers ( $P < 0.01$ ) while ADP zones and educational level were significant ( $P < 0.01$ ) for extension personnel. Regression analysis revealed that the use of ICT had significant impact in fish revenue of fisher folks ( $P < 0.01$ ) but was not significant for fish farmers ( $P > 0.05$ ).

Challenges concerning the use of ICTs as identified by fisher folks, fish farmers and extension personnel include lack of fund, inadequate electricity and low knowledge of ICTs technology. It was recommended that there is a need to encourage the use of Information and Communication Technology among fisher folk and fish farmers by supplying cheap and durable communication tools (phone).

## THE LOW-PRODUCTIVITY SYNDROME OF TILAPIA IN SUB-SAHARAN AFRICA: SHALLOW PONDS, IN-POND REPRODUCTION AND POOR AQUAFEDS IN GHANA

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Pond-based aquaculture in sub-Saharan Africa has been plagued by low productivity. Many causes of this low productivity in tilapia ponds have been identified, but there is no consensus on the most important causes. To optimally allocate development and production effort the multiple, tangled causes should be better understood. Factors commonly cited for the low productivity across countries include low-quality feed, lack of quality fingerlings, poor water quality management, inadequacy of control of reproduction in ponds, and poor stocking practices. Also, frequently unaccounted for is improper pond construction and maintenance. Using data from farmer surveys, farm visits and pond mapping, and multiple on-farm experiments in Ghana, the goal of the current study was to synthesize and tease apart the sources of variation in growth of tilapia and productivity at two scales — the farm and ponds nested within farms.

A combination of grow-out data from four separate experiments across 10 farms and 77 ponds, survey of management practices and farmer-reported annual production from 20 farms, and mapping of over 80 ponds from the corresponding farms, provided a comprehensive dataset for synthesis. Analysis of variance, regressions, and variation partitioning through redundancy analysis isolated the leading factors accounting for growth at the pond-scale and productivity and production at the farm-scale.

Harvest size of Nile tilapia *Oreochromis niloticus* exceeding 350g and productivity exceeding 4,000 kg ha<sup>-1</sup> yr<sup>-1</sup> from ponds were rare. Besides feed quality and feeding rate, which were experimentally controlled, average pond depth emerged as the single most important predictor of productivity at both the pond and farm scales, limiting carrying capacity by over 50% in some cases. Farm-scale productivity increased from 1,000 kg ha<sup>-1</sup> yr<sup>-1</sup> when ponds averaged 0.4 m water depth to > 5,000 kg ha<sup>-1</sup> yr<sup>-1</sup> when ponds averaged 1 m. The use of pond area for dosing lime and fertilizers led to a higher primary production (as measured by chlorophyll-*a*) in shallower ponds, but that did not translate into a higher production of tilapia. Growth rates of tilapia in ponds fed a common home-made feed were only between 0.33-0.50 the growth rates of those fed commercial feeds of similar crude protein content. Unexpected in-pond reproduction frequently interrupted grow-out cycles and led to smaller average harvest size.

Although it is widely believed in Ghana that better-growing tilapia strains are needed to boost aquaculture production, the results of this study suggests that numerous management practices, especially improved pond construction and maintenance, adoption of good commercial feeds and optimal feeding strategies, and control of in-pond reproduction, could more than double current production levels of many ponds and farms. Government and private extension providers need to emphasize better management practices in Ghana and probably across sub-Saharan Africa.

## A NATURAL GUT ENHANCER TO IMPROVE RESISTANCE OF AQUATIC FARMED SPECIES AGAINST PATHOGENIC PRESSURE

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Nowadays, the great challenge of the aquaculture industry is to maximize yield production to meet the customer demand. It induces diseases outbreaks through intensification, introduction of new pathogens through species diversification and pathogens spreading through poor-managed farming systems. This pathogenic pressure significantly impacts the economics of the farmers.

Lately, chemical solutions such as antibiotics have been applied widely. Unfortunately, prophylactic treatment with antibiotics in aquaculture became an increasing and significant public health concern on an international scale: various species developed an antibiotic resistance and dramatic effects on the environment appeared.

A global approach of a active researches is done on this subject to explore natural alternatives to antibiotic treatments.

Various experimentations have been successfully conducted to evaluate the potential of a natural phytobiotic called A-LIVE (supplied by MiXscience, France) which eliminate a broad spectrum of pathogens in aquaculture systems. The antimicrobial effects of the phytobiotic have been investigated both *in vitro* and *in vivo*.

*In vitro*, A-LIVE shows a wide bactericidal action spectrum since it exhibits high efficiency against both gram positive and gram negative bacteria. It also shows (i) a strong antimicrobial activity compared to potential competitor products, (ii) a minimal inhibitory and bactericidal concentrations for both the product and the antibiotics. The potential of A-LIVE is promising as it is proved to be an efficient alternative to antibiotics.

Based on these *in vitro* results, the product has been applied in *in vivo* challenge trials where the targeted species are either freshwater and seawater fish or shrimp.

Overall, a significant reduction (minimum 18% in fish and 40% in shrimp) of mortality has been observed regardless the farmed species and associated pathogens.

We conclude that this new feed additive provides efficient control against a wide range of microbial pathogens and can be considered as an operational, universal and natural solution to reduce the use of antibiotics in aquaculture systems.

## IN SITU MEASUREMENT OF THE DRAG COEFFICIENT OF MESH WITH VARYING LEVELS OF BIOFOULING

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Drag force measurements acting on a mesh sample is typically conducted in a tow-tank. This makes it difficult to take measurements on samples with live biofouling on them since the fouling is disrupted by air exposure and fresh water. Swift *et al.* (2006) developed a protocol for measuring drag forces on fouled mesh which, while effective, requires significant time and resource investment. In an effort to enable regular field-testing, a similar and more easily replicable protocol was developed by InnovaSea to measure the drag force on a mesh sample with biofouling. The protocol uses the known buoyant force of a buoy to drag mesh vertically through the water column. This protocol was used to develop a relationship between the level of biofouling and the drag coefficient of the sample.

A computer algorithm was developed to calculate the solidity ratio of the fouled mesh samples. Solidity ratio is the ratio of blocked vs unblocked areas of the mesh sample. With this tool, farmers using a calculated solidity ratio from a photograph and the local current data, can approximate the drag forces on the pen in near real time.

This work demonstrates a rigorous and easily replicable method for empirical determination of drag forces on fouled mesh at remote sites. The sizable increases in drag forces observed highlight the need for effective fouling management strategies. Submersible pens offer a unique advantage as they can be submerged below the thermocline during the summer for cold water species, and to lower light intensity layers of the water column. These pens also can be raised out of the water to desiccate the mesh providing a low effort method of cleaning and killing parasites.

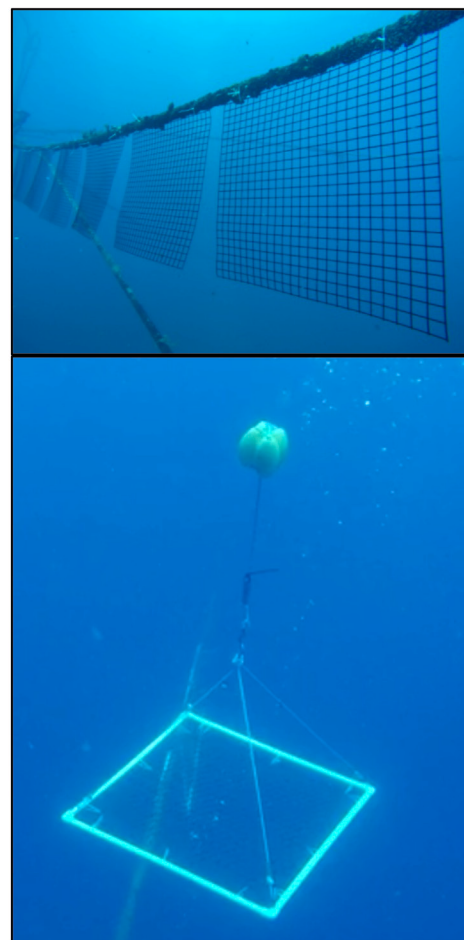


Figure 1 : Top: Newly deployed mesh panels will foul over time in the water. Bottom: The in situ drag test apparatus in action.



## GENETIC LINE AND INBREEDING EFFECTS FOR SURVIVAL TIME AND SURVIVAL RATE IN PACIFIC WHITE SHRIMP *Penaeus vannamei*

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The objective of this study was to compare survival time at 50% in experimental challenges to Acute Hepatopancreatic Necrosis Necrotic Disease (AHPND), between two genetic lines, a line with a history of selection for growth and survival rate with no disease outbreaks (Growth), and a line created from a mixture of shrimp with a history of resistance to White Spot Disease (Resistance). In addition, shrimp created by sib-mating allowed the estimation of inbreeding (F) effects.

Two experimental challenges to AHPND were performed in Mazatlán, Sinaloa during 2015 and 2016. Shrimp were inoculated with a culture of the strain M0904 of *Vibrio parahaemolyticus* at infecting doses. Data for survival rates during the grow-out period (approximately 70-130 days of age), in two ponds in 2015, and in three ponds in 2016 were also obtained. Data involved shrimp from both lines and crossbreeds. Total number of families were 182 in 2015 and 212 in 2016. Number of observations per line in the experimental challenges varied from 1477 to 2470. Number of observations per line on each pond varied from 1063 to 3176. Analysis of data was performed using fixed-effects linear models which contains crossbreeding and inbreeding effects.

Predicted values for noninbred animals in survival time at 50% favoured the Resistance line in 2015 ( $P < 0.05$ ). Predicted values for inbred animals were higher or similar to those for non-inbred animals (Table 1). The results of survival rates in the grow-out ponds with a disease outbreak showed a superiority of the Resistance line over the Growth line, compared to that found in ponds without disease outbreak ( $P < 0.05$ ) (Table 2).

Survival rates decreased 1% per 10% increase in F ( $P = 0.003$ ) in pond 2016-1, and in pond 2016-2 ( $P = 0.03$ ) in both lines. No inbreeding effect was found in the other ponds.

Table 1. Average predicted survival times at 50% to challenge tests for AHPND in 2015 and 2016 for Resistance and Growth lines at two inbreeding (F%) levels.

| Line       | Year          |             |              |              |
|------------|---------------|-------------|--------------|--------------|
|            | 2015          |             | 2016         |              |
|            | F% = 0        | F% = 25     | F% = 0       | F% = 25      |
| Resistance | 8.52 ± 0.05 A | 8.67 ± 0.20 | 59.65 ± 0.97 | 63.77 ± 1.54 |
| Growth     | 8.02 ± 0.11 B | 8.86 ± 0.29 | 57.46 ± 1.10 | 59.34 ± 2.17 |

Means in the same column with different letters (A, B) are different ( $P < 0.05$ ).

Table 2. Average survival rates in grow-out ponds for Resistance and Growth lines.

| Line       | Pond*        |              |              |              |              |
|------------|--------------|--------------|--------------|--------------|--------------|
|            | 2015-1 (+)   | 2015-2 (-)   | 2016-1 (+)   | 2016-2 (-)   | 2016-3 (+)   |
| Resistance | 58.78±0.74 A | 73.04±1.35 A | 32.57±0.71 A | 81.24±0.67 B | 57.49±0.75 A |
| Growth     | 3.68±0.77 B  | 61.81±1.41 B | 2.88±0.71 B  | 88.49±0.82 A | 8.56±0.88 B  |

Means in the same column with different letters (A, B) are different ( $P < 0.05$ ).

\* (+) = pond with a disease outbreak; (-) = pond without disease outbreak.

## GROWTH OF SANDFISH *Holothuria scabra* JUVENILES DURING AN ENSO

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A demonstration of large-scale pond cultivation of sandfish (*Holothuria scabra*) in Vietnam recorded average mean temperatures which ranged from 27.4°C-29.5 °C  $\pm$  3.4. The same pond culture may present local nuances in the Philippines which is a segment of the eastern border of the Pacific Ocean. With aggravating climate change, earth ponds in this country are relatively susceptible to increasingly unpredictable weather disturbances, among them the El-Nino Southern Oscillation (ENSO). In the last two decades, the Philippines, has experienced more frequent ENSO. This study presents the growth performance of sandfish juveniles reared in a pond during a moderate ENSO in 2009-2010.

A sequential growth experiment was done: October 2009 to February 2010 then January to April 2010. For October, a 15m x 10m polyethylene pen was laid out in a marine earthpond. Onto it, 150 juveniles, averaging 5.74g were introduced. For January, 100 juveniles, averaging 10.82g were used. Using a water logger, the mean bottom temperature at 6:00PM ranged from 31.5°C-35°C and 34.0°C-42°C for the October and January batches, respectively. One-way ANOVA ( $p < 0.05$ ) showed that the growth increment in the October batch was significantly higher every month with a 100% survival in the third pen (Fig.1). Growth in the January batch was significant only in the first month. By April when the bottom temperature did not go lower than 37°C, a total of only 10 individuals were recaptured with two showing skin 'melting'. Two-way ANOVA ( $p < 0.05$ ) showed that sustained high temperatures of the pond brought about by the 2010 El Niño correlated with the mortality of the juveniles. In the Philippines context, temperature variation during an ENSO must be carefully monitored and managed when growing out sandfish in ponds.

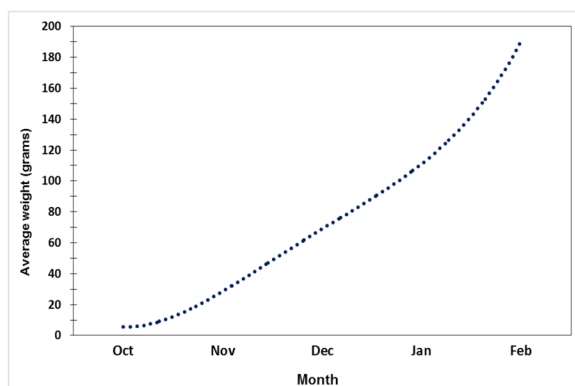


Fig. 1. Growth of juveniles in a marine pond before the onset of El Niño.

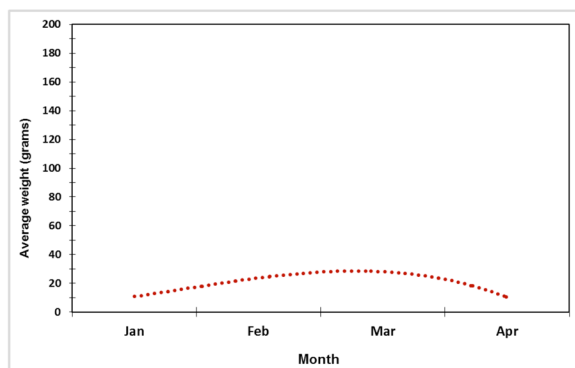


Fig. 2. Growth of juveniles in a marine pond during an El Niño.

## THE ROLE OF DIAGNOSTIC LABORATORIES AND DIAGNOSTIC TESTS IN AQUACULTURE DISEASE SURVEILLANCE

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Irrespective of species, diagnostic testing laboratories aim to provide reliable results for a specific pathogen and diagnostic test combination that are useful to assist decision-making of end-users (e.g. veterinarians). To achieve this aim, most laboratories have a quality management system and standard operating protocols (SOP) including use of samples of known status (positive and negative) in a regular basis to provide confidence that tests are operating acceptably. National laboratories are often accredited by standards-setting bodies such as the International Standards Organization. Participation in ring and proficiency testing rounds provides further assurances that laboratory results are reproducible. The range of offered tests differs between national and state/provincial laboratories and OIE reference laboratories are willing to provide support for diagnostics, if not available in country. For OIE-listed diseases of fish, molluscs, crustaceans and amphibians, the OIE Manual of Diagnostic Tests for Aquatic Animals ([www.oie.int/international-standard-setting/aquatic-manual/access-online](http://www.oie.int/international-standard-setting/aquatic-manual/access-online)) provides guidance on recommended tests. Current work by an OIE *ad hoc* group involves development of a new chapter template that provides more explicit information on the diagnostic sensitivity and specificity of currently-used tests. Case definitions are being added to disease-specific chapters as they are updated and these definitions are based on diagnostic test results from 2 independent tests and epidemiological information such as mortality and clinical signs. The OIE Manual (chapter 1.1.2) provides guidance on validation of tests. The robustness of the laboratory results depends greatly on correct sample selection (animal and tissue), appropriate handling and preservation in the field, and timely transportation to the laboratory. This is more critical for some tests (e.g. bacterial, parasite and virus isolation) than others (e.g. PCR) but in principle, best practices must be followed to give the testing laboratory the highest probability of diagnostic success. There is increasing use of pond-side tests for presumptive diagnosis (e.g. white spot syndrome). Use of multiplexing in the receiving laboratory now provides the opportunity to test for multiple pathogens on the same specimen type and next generation sequencing is playing an increasing role where known pathogens cannot be detected and gross and histopathology are highly suggestive of an infectious agent.

Few pathogens of aquatic animals have published DSe data for surveillance purposes. In a true surveillance scenario, where apparently healthy but infected animals are tested it is critical that the diagnostic sensitivity (DSe) and specificity (DSp) are evaluated. Because loads of the target organism or analyte are lower than in an outbreak scenario, the DSe values might be substantially reduced compared to outbreaks where DSe is often >95%. An example of *Francisella noatunensis subsp. orientalis* in Nile tilapia in Brazil will be presented to demonstrate how the DSe of bacterial isolation and qPCR change in different scenarios. The net effect of the lower DSe is that more samples need to be tested to establish a diagnosis and some tests (e.g. bacterial isolation) may have such low DSe that their use is unlikely to give positive results.

## DIETARY AMINO ACID REQUIREMENTS OF THE RED DRUM *Sciaenops ocellatus*

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In recent years there has been increased efforts to identify feedstuffs to potentially replace some portion of fishmeal in the diets of various aquatic species, thereby increasing the sustainability and cost-effectiveness of aquaculture. However, many alternative protein feedstuffs are deficient in one or more indispensable amino acids (IAAs). Thus, there is a critical need to establish refined estimates of IAA requirements of various species produced in aquaculture. Therefore, this laboratory intensified its efforts in recent years to quantify the dietary requirements of red drum (*Sciaenops ocellatus*) for various IAAs. This species is produced intensively in the United States and in some Asian countries as a popular seafood as well as in the Gulf Coast region of the U.S. for stock enhancement of recreational fisheries.

A series of feeding trials were conducted in which the experimental diets were formulated to contain 35% crude protein (CP) and 3.2 kcal digestible energy/g by combining lyophilized red drum muscle (RDM) as an intact protein (10.5% of dietary protein) and supplementing with crystalline L-AAAs to simulate the pattern found in 35% CP from RDM. In each trial, graded levels of the amino acid under investigation were provided in crystalline form, and the experimental diets were maintained isonitrogenous by adjusting the inclusion of an aspartate/glutamate premix. The feeding trials were conducted in glass aquaria connected as recirculating systems to maintain optimal water quality and salinity at ~7 ppt. Juvenile red drum of the same parentage and initially weighing less than 10 g/fish juveniles were used in the trials, and diets were fed to fish in triplicate aquaria at a rate approaching apparent satiation, twice daily, for 6 to 8 weeks. Minimum dietary requirement estimates were obtained by subjecting weight gain, feed efficiency and protein conversion efficiency data to quadratic broken-line regression analysis. Table 1 summarizes requirement values determined to date for red drum.

**Table 1** Quantitative dietary amino acid requirements of red drum.

| Amino acid             | Requirement   |                      | Reference                 |
|------------------------|---------------|----------------------|---------------------------|
|                        | % of dry diet | % of dietary protein |                           |
| Arginine               | 1.80          | 5.14                 | Barziza et al., 2000      |
| Histidine              | 0.89          | 2.50                 | Peachy and Gatlin, 2016   |
| Isoleucine             | 1.10          | 3.14                 | Castillo and Gatlin, 2016 |
| Leucine                | 1.57          | 4.48                 | Castillo and Gatlin, 2016 |
| Lysine                 | 1.55          | 4.43                 | Craig and Gatlin, 1992    |
| Methionine+Cystine     | 1.20          | 3.43                 | Moon and Gatlin, 1991     |
| Phenylalanine+Tyrosine | 2.10          | 6.00                 | Castillo et al., 2015     |
| Taurine                | 1.00          | 2.86                 | Velasquez et al., 2015    |
| Threonine              | 0.80          | 2.28                 | Boren and Gatlin, 1995    |
| Tryptophan             | 0.28          | 0.80                 | Pewitt et al., 2016       |
| Valine                 | 1.22          | 3.48                 | Castillo and Gatlin, 2016 |

## FISH CONSUMPTION IN URBAN LUSAKA: AQUACULTURE YET TO SUPPLY THE POOR

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The main sources of consumed fish in Zambia derive from natural fisheries and aquaculture production and international imports. For many years outputs in Zambian fisheries have stagnated while the population growth and demand for fish are continuously increasing. This trend has recently resulted in a decline in per capita fish supply, which is worrying in terms of food and nutrition security, and especially for the poor who often suffer from under- and / or malnutrition. As fish is known as a comparatively healthy animal-source protein food also rich in essential fatty acids and micronutrients, increased availability and accessibility of fish among the poor is expected to make an important contribution towards improved health outcomes. Among the various sources for and current trends in fish supply, aquaculture is considered to have the most potential to increase per capita fish supply in Zambia. However, given recent changes in demography and fish supply, little is known about the actual role of farmed fish in the diets of resource-poor people. The aim of this research was to generate information on fish consumption patterns amongst poor consumers in urban Zambia based on their sex, age and socioeconomic status. In addition, the research wanted to understand the role of aquaculture as a source of fish among poor consumers in urban areas. The research was based on a cross-sectional study where a quantitative household survey (N=714) was used to assess fish consumption patterns (including species, quantities, size (small vs large fish), products (fresh, dried, etc.)) through a 24-hour food recall and a 7-day household dietary diversity recall. Data on household assets were collected and used to calculate an asset-based wealth index (*Principal Component Analysis* (PCA)) to enable the comparison of fish consumption patterns among different wealth groups.

The results of the study show, for example, that the consumption of fish species and sizes are strongly (and statistically significant) associated with wealth. Resource-poor households largely rely on smaller-sized and often dried fish. Instead, relatively wealthier households show frequent consumption of larger-sized, fresh Tilapia (or *bream*) (see Table 1).

Based on the species composition in the diets of sampled households we argue that the aquaculture sector in Zambia (almost exclusively producing fresh Tilapia of >400 grams per piece) does not yet supply the urban poor, who largely rely on fish from natural fisheries. Unlike better-off households, who can afford to supplement their fish demand with new sources and products, resource-poor households may lack alternative means to maintain or even increase consumption of larger farmed fish, which could have negative consequences on their food and nutrition security given the declining fish supply from natural fisheries in Zambia. To avoid this scenario, we conclude that the aquaculture sector needs to further expand their economies of scale leading to larger production, lower fish prices and thus making fish available and accessible to resource-poor households. Further, given the finding that resource-poor households largely consume smaller-sized fish, which we argue is due to comparatively lower prices and high divisibility in retail, the production of smaller-sized fish (harvesting juveniles of larger fish species and / or cultivation of small species) in aquaculture might be another possible innovation for commercial aquaculture entering the bottom of the pyramid market and thus possibly enhancing their role in food and nutrition security for the poor in Zambia.

| Fish species consumed last 24h | WG 1<br>n (%) | WG 2<br>n (%) | WG 3<br>n (%) | WG 4<br>n (%) | P (P. Chi-Square) | Total<br>n (%) |
|--------------------------------|---------------|---------------|---------------|---------------|-------------------|----------------|
| Kapenta                        | 38 (70.4)     | 26 (44.8)     | 17 (39.6)     | 23 (32.0)     | .011              | 104 (45.6)     |
| Bream                          | 12 (22.2)     | 21 (36.0)     | 18 (41.9)     | 35 (48.6)     | .009              | 86 (38.0)      |
| Buka Buka                      | 3 (5.6)       | 3 (5.1)       | 2 (4.7)       | 3 (4.2)       | .973              | 11 (4.8)       |

Table 1: Selected frequencies of fish species consumed by households 24h prior the interview

## CONCEPT AND FIRST EXPERIMENTAL STUDIES FOR THE CULTIVATION OF SALINE MICROALGAE IN INLAND SYSTEMS USING GEOTHERMAL WATER RESOURCES

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After the termination of black coal mining activities in the Zwickau district in 1978 the up to 1200 m deep mine workings were flooded until the end of 1995. In recent past different activities were started to investigate geological and hydrological aspects in the area as well as to estimate the potential of applying geothermal water resources for heating of non-residential buildings. In 2014 a deep drilling was completed about 500 m from the city centre of Zwickau, Germany. From a depth of more than 625 m geothermal water is pumped to ground level. This water has average temperatures of 22...25 °C and is therefore intended for the heating of several buildings of University of Applied Sciences Zwickau applying low-temperature heating grid and decentralized heat pump system (100 kW thermal power; average flow rates of 50...70 m<sup>3</sup>/h).

The chemical properties of the geothermal water were characterized in detail. Chlorine concentrations were 16...23 g/l, while sodium and calcium concentration was found between 5.4 g/l and 6.7 g/l or 3.8 g/l and 5.4 g/l, respectively. Whether the saline water might be discharged to the onsite pre-flooder (river Mulde) without further treatment is questionable, because of several ecological concerns. On the other hand the water is containing some iron compounds as well as nitrate and sulfate, only very low concentrations of heavy metals and no organic pollutants (like PAH, PCB and CHC).

The aim of a joint research project was to develop and estimate different concepts for the further utilization of the water resources after the use of the thermal potential (remaining temperature between 12 °C and 18 °C). Besides the separation of salt components in a kind of brine graduation tower the cultivation of saline microalgae (e.g. *Nannochloropsis* sp.) is an opportunity for the further use of the chemical potential of the geothermal waters. These species are typically cultured e.g. as a fish feed component due to the contained fatty acids. There have been only very few comparable approaches in the last decades, which nevertheless gave a principal proof of concept (cf. [Rastegary et al., 2013]).

Different concepts for bioreactor construction (e.g. air-lift flat panels for wall-installation) and energy efficient fluid circulation are presented as well as first results from cultivation experiments for different algae species in laboratory scale applying standard media and (artificial) geothermal waters with different nutrient supplements.

RASTEGARY, J.; SHIRAZI, S. A.; FERNANDEZ, T.; GHASSEMI, A. (2013): Water resources for algae-based biofuels. Journal of Contemporary Water Research & Education **151**, pp. 117-122.

## **POST-EXTRUSION APPLICATION OF EMULSIFIED ENDO-1,4-Beta-XYLANASE IN DIETS OF AFRICAN CATFISH *Clarias gariepinus***

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The application of arabinoxylan degrading enzymes is a well-established practice in commercial pig and poultry nutrition feeds. Xylanase enzymes typically help minimise the antinutritional effect of soluble arabinoxylans while facilitating the release of energy from insoluble arabinoxylans. However, due to the heat-sensitive nature of these enzymes, its application in extruded aquafeeds is limited due to temperatures typically exceeding 110°C. The installation of post-pelleting or post-extrusion applicators is often a costly exercise and inclusion through existing post-extrusion oil application systems (i.e. fish oil coating) may be a more practical option.

A 90-day trial was designed evaluate an Endo-1,4-Beta Xylanase (Nutrase Zyttest L1, supplied by Nutrex Belgium) and a lecithinated vegetable oil (Lesitol, Nutritionhub South Africa), emulsified in fish oil on zootechnical performance of African catfish. Treatments consisted of a 1) control diet containing 6% fish oil, diets of which 10% of the control diet's fish oil was replaced with 2) non-emulsified vegetable oil and 3) lecithinated vegetable oil, diets containing 4) 100 ppm and 5) 200 ppm liquid xylanase applied by conventional post pellet spray method, and diets containing 6) 100 ppm and 7) 200 ppm liquid xylanase emulsified with the 10% lecithinated vegetable oil portion of Diet 3. Each of the 7 treatments were replicated six times. Results will be presented.



## AQUACULTURE INVESTMENT OPPORTUNITIES & INCENTIVES IN SOUTH AFRICA

Department of Agriculture, Forestry and Fisheries (DAFF): Ms Lisa Geswindt, Deputy Director: Investment Promotions Aquaculture Operation Phakisa Delivery Unit; and the Department of Trade and Industry (DTI): Mr Brian Soldaat, Director: Invest SA.

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Operation Phakisa is an initiative of the South African government which aims to implement priority economic and social programmes better, faster and more effectively. The Operation Phakisa: Oceans Economy was launched by His Excellency President J.G. Zuma, in October 2014.

Aquaculture led by the Department of Agriculture, Forestry and Fisheries is one of the six growth areas prioritised to contribute to unlocking the economic potential of South Africa's oceans. This was based on its potential contribution to economic growth and job-creation.

The first year of implementation of the Aquaculture Operation Phakisa saw good progress in terms of its contribution towards economic growth (R29 million) and the total committed investment into the sector was R444 million from the private sector and government. However, there has been a growing need to build and enhance capacity particularly in light of the very limited enabling resources (e.g. budget).

The initiatives and projects listed on the Aquaculture Operation Phakisa dashboard require significant investment, along with improving awareness and access to markets. Investment promotion is key to meeting the target set over the next five years (2014-2019) to grow sector revenue from R0,67 billion to R3 billion and ensure an increase of jobs from 2227 to 15 000.

The aquaculture investment opportunities available are in the form of Aquaculture Development Zones located in various provinces as well current farms registered on the Aquaculture Operation Phakisa which require investment. An Aquaculture Development Zone (ADZ) is an area or site either on land or sea, set aside exclusively for aquaculture use or development. An ADZ may have bulk infrastructure such as a reservoir or water pump to attract investors and these zones are supported by key government policies such as the Industrial Policy Action Plan (IPAP); the draft Agricultural Policy Action Plan and the National Aquaculture Policy Framework.

Investment South Africa (InvestSA) is a division of the Department of Trade and Industry and provides a one-stop-shop service to investors. The core mandate of InvestSA is to increase the quality and quantity of foreign and domestic direct investment into South Africa, by marketing and promoting investment into key high yielding growth sectors of the South African economy. The Department of Trade and Industry offers the following incentives for the aquaculture sector:

1. Aquaculture Development and Enhancement Programme (ADEP)
2. Critical Infrastructure Programme (CIP)
3. 12I Tax Allowance Incentive

## CONCENTRATING WHITE SPOT SYNDROME VIRUS BY SKIM MILK FLOCCULATION FOR DETECTION BY A MONOCLONAL ANTIBODY BASED FLOW-THROUGH ASSAY

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Since its emergence, white spot syndrome virus has caused huge loss to shrimp aquaculture. Although early detection of viruses in water is a prerequisite to evade epizootics but the greatest obstacle is that they are present in very low copy number and hence, concentrating them is very much essential. To address this, the present study was conducted in seawater under acidic (pH 3.5) and neutral (pH 7.4) conditions to concentrate WSSV by a one-step concentration method using skim milk (0.01%) as an organic flocculent and detection by a MAb based flow-through assay, RapiDot. This method under *in-vitro* conditions was developed and standardized to concentrate WSSV VP28 which was detectable by RapiDot in both subsurface and floc samples. WSSV concentrated from seawater in 2 L, 1 L and 50 mL from the shrimp infection experiment was tested where RapiDot detected WSSV earlier compared to 1-step PCR in floc samples and overall acidified seawater in 2 L gave the best results. However, concentrating WSSV in seawater under normal pH which is simple than acidified environment equally performed well and is recommended for field level.

A 36 h duration study was conducted in seawater under acidic (pH 3.5) and normal (pH 7.4) conditions to concentrate WSSV by a one-step concentration method using skim milk (0.01%) as an organic flocculent and detected by a MAb based flow-through assay, RapiDot. This method was developed and standardized under *in-vitro* conditions to concentrate WSSV VP28 for detection by RapiDot and PCR in both subsurface and floc samples. WSSV concentrated from seawater in 2 L, 1 L and 50 mL from the shrimp infection experiment was tested by RapiDot and PCR.

WSSV VP28 was detectable by RapiDot in both subsurface and floc samples. WSSV concentrated from seawater from the shrimp infection experiment was detected earlier by RapiDot compared to 1-step PCR in floc samples and overall acidified seawater in 2 L container was the best. However, concentrating WSSV in seawater under normal pH which is simple than acidified environment equally performed well and is recommended for field level.

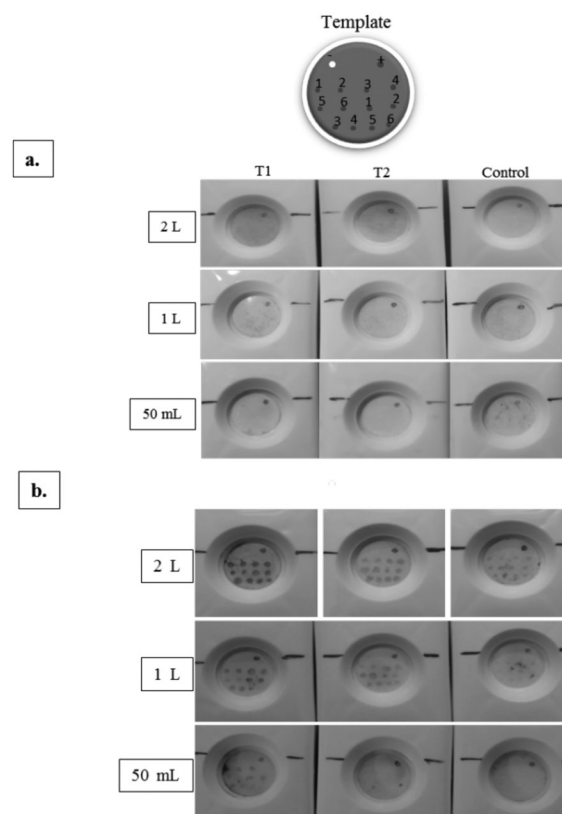


Fig. 1. RapiDot analysis of WSSV flocculated by skim milk in subsurface and floc samples from the experimentally infected water. Appearance of blue dots showing the presence of WSSV in subsurface (a) and floc (b) samples of T1, T2 and control from 2L, 1 L and 50 mL. The order of samples corresponds to the sampling at different intervals of time from 6, 12, 18, 24, 30 and 36 h, are depicted as numbers in the template.

## PERIPHYTON BASED DIFFERENT APPROACHES IN FISHERIES MODULES: IMPLICATION OF SUSTAINABLE LIVELIHOOD SECURITY OF THE FARMERS IN RURAL SUNDERBAN OF INDIA

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The study was conducted to assess the alternative livelihood conditions through periphyton technology in different aquaculture practices in vast area in Sundarban by using semi-structured and pre-tested interview schedule in order to assess the alternative livelihood for rural youth men and women empowerment. Periphyton is the complex of sessile aquatic biota with associated detritus, attached to submerged substrates. It includes phytoplankton, zooplankton and other bottom organisms in combination with microbial bio-films.

About 49% of farmers of South 24 Parganas are small and marginal. A possibility of regular income generation through traditional farming is limited due to small land holding, soil salinity, frequent attacks of cyclones like 'Aila', floods, sea water intrusion, agricultural practices without scientific knowledge and crop failures due to pests and diseases. The district is exposed harsh agro-climatic situations leading to frustration, abandoning farming, migration of farming communities seeking better livelihood opportunities, shift to alternate income earning opportunities etc. Most of the farm-families in the district have one or more than one pond in and around their dwelling areas. These ponds and its surrounding areas may be a major source of income to sustain their livelihood if utilized efficiently in a scientific manner. But the reality is that in most of the cases these important resources remain unutilized without any considerable income due to lack of awareness, technologies and examples before them. In this backdrop, 'different approaches in fisheries modules approach is envisaged as an effective tool to create examples before the farmers so that they become able to tap their own resources for sustainable income generation and to enhance their livelihood security. The units may be of different types depending on the resources and capacity of the farmers. Considering superior periphyton growth on bamboo posts, most production trials were carried out with bamboo as substrate. This material is too expensive in South-Asia for resource poor farmers. Therefore, cheap alternatives for the bamboo substrate are sought. The minimum components of different approaches in fisheries modules will be- i) mono culture (23%), ii) poly culture (52%) and iii) brackishwater farming (25%).

**Mono culture:** Scampi culture with periphyton technology, monosex tilapia farming, mud crab culture and fattening, bhetki farming.

**Poly culture:** Fish culture with IMC- Rohu: Catla:Mrigel:, magur farming with bhangor fish, *Anabus* with scampi.

**Air-breathing fish culture-** Culture of Magur, Koi or Singhi either single species or in combination

**Increasing and popularizing of indigenous species farming**

## MORPHOMETRIC AND BEHAVIOURAL CHANGES IN THE EARLY LIFE STAGES OF THE SEA CUCUMBER *Cucumaria frondosa*

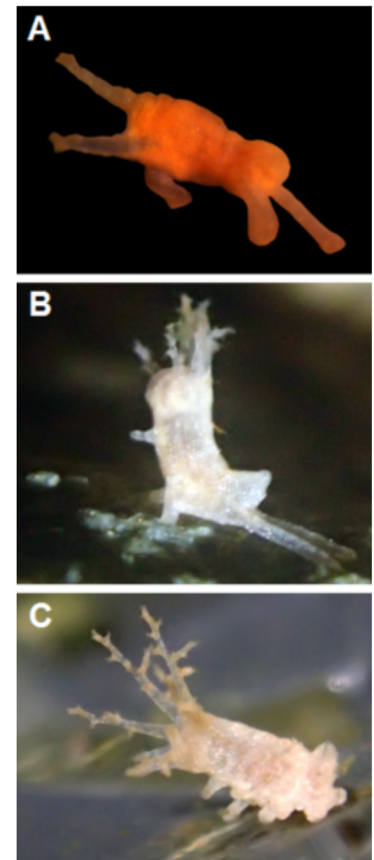
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Developments in nursery rearing of sea cucumber juveniles have benefitted from studies on the developmental biology, ecology, and behaviour of early life stages. Post-settled and early juveniles of sea cucumbers have specific environmental requirements, which might change as individuals continuously grow and develop. The dendrochirotid sea cucumber *Cucumaria frondosa* is widely distributed in cold waters of the North Atlantic and the species has recently been propose to have potential for aquaculture. The present study investigated morphometric changes during early developmental stages as well as behaviour of juveniles under different environmental conditions.

Morphometrics of juveniles of *C. frondosa* were measured monthly for up to 21 months. Behavioural experiments were also conducted to compare changes in response of 1, 6 and 12-month-old juveniles to different conditions of light intensity (0-100 Lux), substrate type (coralline algae, rocks, and shells), background colour (black, white, and red), and water flow regime (0-20 cm s<sup>-1</sup>).

Juveniles continuously grew throughout the experiment, measuring 4.6 mm after 21 months under natural environmental conditions of water temperature and food availability (Figure 1). Individuals had developed all 10 oral tentacles after 12 months, but the size of the tentacles, the number of tentacle ramifications, and the number of ambulacral podia continued to increase. Sensitivity to light decreased and tolerance for high flow increased with age. There was a marked preference for substrates composed of coralline algae and rocks and for dark background in all age classes. A clear understanding of the requirements of juveniles provide essential background for developing aquaculture and stock enhancement programs of cold-water sea cucumber species such as *C. frondosa*.



**Figure 1:** *C. frondosa* juveniles. (A) 1 month old, (B) 6 months old, and (C) 21 months old.

## ENVIRONMENTAL CONTROL OF GAMETE PRODUCTION, SPAWNING AND EGG QUALITY IN THE SEA CUCUMBER *Cucumaria frondosa*

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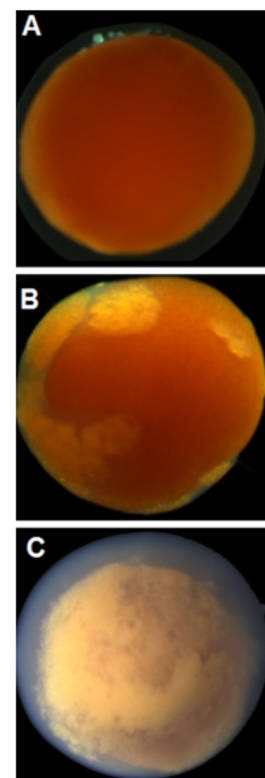
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Understanding how environmental parameters modify reproductive cycles, synchronize spawning and affect egg quality is fundamental to the successful management of broodstock in captivity. The dendrochirotid sea cucumber *Cucumaria frondosa* is widely distributed in cold waters of the North Atlantic and has recently been identified as a candidate species for aquaculture due to its market value for seafood and nutraceutical products. Here, we investigated the influence of various environmental parameters on gamete production, spawning and oocyte/egg quality in *C. frondosa*.

Adult sea cucumbers were conditioned under different water temperature (6 and 12°C) and light regimes (24-h dark, 24-h light and advanced photoperiod) for a total of 4 months prior to the spawning period. They were compared to control individuals kept under ambient environmental conditions (naturally fluctuating water temperature and light regime). Sea cucumbers were sampled for gonad indices, fecundity and maturity at the onset of the experiment, as well as before and after the estimated natural spawning time for *C. frondosa*. When spawning occurred, eggs were collected and assessed for fertilization rates and quality.

Sea cucumbers from all treatments presented comparably high fecundity before the spawning time. Spawning occurred on the same day in all control tanks, which yielded the highest number of good-quality eggs (Figure 1a). In treatments which light regimes were manipulated, spawning occurred only in 1 or 2 of 3 tanks and high proportions of either damaged or decaying eggs were observed (Figure 1b and 1c). No spawning occurred in tanks kept at 6 and 12°C.

As aquaculture initiatives expand to cold-water sea cucumbers, understanding how environmental parameters control gametogenesis and spawning in captivity is fundamental for successful production.



**Figure 1:** Phenotypes of *C. frondosa* eggs. (A) Normal fertilized egg, (B) egg with abnormal cytoplasm, and (C) decaying egg.

## NEW INSIGHTS IN THE USE OF SPRAY-DRIED PLASMA (SDP) IN FISH DIETS

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There is a global recognition that terrestrial animal by-product meals, including blood meal and blood products, represent a largely untapped source of animal proteins and lipids for the aquafeed industry. Spray-dried blood and plasma proteins are recognized as safe, high-quality feed ingredients for farmed animals. SDP has an excellent AA profile and close to 99% digestibility and due to its immune modulatory components, including immunoglobulins, bioactive peptides and growth factors, SDP has been recommended for improving production during stressful events, as well as a source of immunological support. While there is much research on the use of SDP in terrestrial animals, little is known about the use of this feed ingredient in aquafeeds.

Previous data in sea bream (*Sparus aurata*) showed that SDP benefited fish by enhancing intestinal and serum innate immune functions, modulating the activity of antioxidative stress enzymes in the intestine and promoting growth performance. Thus, in this study authors wanted to provide further insight into the effects of SDP in fish by evaluating the addition of SDP at 3% (AP820P; APC Europe SL, Spain) in diets containing 51% protein and 17% lipids (20.6 MJ/kg GE). SDP was incorporated into the diet at the expense of pre-digested fish meal (CPSP 90). The experimental diet was compared to fish fed with control diet (4 replicates) using the following parameters: growth, HSI and VSI, fillet composition (proximate composition and fatty acid profile), gut microbiome (DGGE DNA profiling followed by determination of the microbial DNA sequences of the 16S rRNA gene by Illumina® technology; sequence analysis using CLcommunity™ software, ChunLab) and the transcriptomic profiling of the skin, intestine and head kidney (HK) using a 4x44K oligo microarray (Agilent Technologies).

Results showed that the administration of the SDP diet increased growth performance ( $88.2 \pm 1.6$  g vs.  $82.7 \pm 3.2$  g) when compared to the control diet. SDP did not affect the HSI (1.3%), but it increased the VSI ( $4.4 \pm 0.1\%$  vs  $4.0 \pm 0.1\%$ ) and reduced the level of perivisceral fat ( $1.1 \pm 0.1\%$  vs  $1.3 \pm 0.1\%$ ). It also reduced the lipid content in fillets ( $8.2 \pm 0.4\%$  vs  $11.1 \pm 0.7\%$ ) without affecting their fatty acid profile. In addition, SDP resulted in changes in the gut microbiota. The transcriptomic response showed a statistically significant tissue-dependence expression pattern ( $P < 0.05$ ) in fish fed with SDP compared to the control group, with 816 differential expressed genes (DEG) in intestine, 194 DEG in skin, and 66 DEG in HK. These results are presented and discussed with regard to the potential use of this ingredient in fish diets.

This study was funded by Ministerio de Economía y Competitividad (Gobierno de España) by means of the project AGL2014-51836-C5-5-R.



## **LARVAplus: AN INTEGRATIVE RESEARCH NETWORK FOR PROMOTING FISH LARVICULTURE IN IBERO-AMERICA**

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LARVAplus is a research and technical network funded by the “Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo” (Programa CYTED) that aims to generate a place for the exchange of knowledge and experience for the benefit of the development of the Ibero-American aquaculture industry. In particular, the network is focused in the production of fish larvae and fingerlings, improving the scientific and technological competitiveness of the sector, promoting the sustainable and responsible growth of the industry, as well as a transversal transfer of knowledge, skills and abilities between the research centers and the industrial sector.

In this context, LARVAplus involves different actors - universities, public research institutes, professionals and hatchery technicians and managers- in the study of the biology and production of fish larvae and fingerlings. Through a multilateral and multidisciplinary framework, LARVAplus will bring new data and approaches to be applied on broodstock management, fish larvae production and quality, water management, as well as larval rearing protocols and feeding strategies to 31 different species (12 freshwater and 19 marine) in order to consolidate and promote Ibero-American aquaculture diversification.

In order to achieve the above-mentioned goals, LARVAplus will organize during its four years of duration (2017-2020): i) different meetings among members of the network, with invited renown speakers on fish larval rearing and broodstock management; ii) funding short research and training missions between members of the network; and iii) organizing open training courses to the industry and scientific community.

The consortium is formed by 15 research institutions (Universities and R+D+T institutes) and 10 private companies from the Ibero-American geography (Spain, Portugal, Peru, Argentina, Chile, Mexico, Colombia, Costa Rica and Brazil).



## REPLACEMENT OF FISH OIL WITH *Schizochytrium* sp. IN RAINBOW TROUT (*Oncorhynchus mykiss*) JUVENILE DIETS: EFFECTS ON GROWTH PERFORMANCE, PROXIMATE COMPOSITION AND HUMORAL NON-SPECIFIC IMMUNE PARAMETERS

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Many studies have been focused on the search for useful functional ingredients for inclusion in aquafeeds, which might reduce the great dependency on conventional ingredients. Thus, the industry has introduced substantial changes in diet formulation during recent years, and strived to decrease the level of ingredients of animal origin in the search for alternatives to fish meal and oil (FO). This search has been mostly directed towards the use of terrestrial plant ingredients; however, relatively little attention has been given to the use of macro- and microalgae biomass in fish diets. In this study, authors tested the partial substitution of FO by *Schizochytrium* sp. in juvenile rainbow trout diets in order to evaluate the possibility of formulating ecologically and socially sustainable aquafeeds, with reduced FO content and without having to switch to vegetable oils from industrially farmed crops.

Juvenile rainbow trouts (initial BW = 75.5 g) were fed three experimental diets (48% proteins, 22% lipids) where FO was substituted at 0, 7.5 and 15% by *Schizochytrium* sp. Diets (feed ratio = 1.5% stocked biomass) and were tested for 45 days at  $17.7 \pm 1.7$  °C. At the end of the trial, fish were sampled for measuring their growth, proximate composition, fillet and liver fatty acid profiles, activity of antioxidative stress enzymes, histological organization of the liver and intestine, and markers of serum non-specific immune response (lysozyme, complement and bactericidal activities, as well as protease and peroxidase activities).

Growth performance indicated that substitution FO by *Schizochytrium* sp. did not affect fish growth in terms of final BW (155.7 – 160.6 g), nor their HSI or VSI. Experimental diets did modify the proximate composition of the fillet and liver, whereas they altered the fatty acid profile by reducing the amount of mono-unsaturated fatty acids and increasing the levels of total n-6 PUFA in both tissues. The inclusion of *Schizochytrium* sp. did not affect the activity of antioxidative stress enzymes (CAT, SOD, GPX, GR and GST) or the levels of lipid peroxidation (TBARS) in comparison to the control group, nor the normal histological organization of the liver and intestine. No changes in the serum non-specific immune markers were observed between trouts fed the control and *Schizochytrium* sp. containing diets. These results confirm previous results in Nile tilapia and Atlantic salmon that showed *Schizochytrium* sp. as an alternative and safe ingredient for fish diets.

This study was funded by the CENIT-ACUISOST project (CDTI, Spain) and IRTA.

## DIETARY LPS DERIVED FROM *Pantoea agglomerans* INFLUENCES THE IMMUNE RESPONSE IN EUROPEAN EEL (*Anguilla anguilla*) ELVERS

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Dietary immunostimulants are considered as an effective tool for enhancing the immune status of cultured organisms. These additives are chemical substances that transiently activate elements of the immune response and may potentially render animals more resistant to infectious diseases, reducing the risk of disease outbreaks, if administrated prior to situations known to result in stress (e.g. handling, change of temperature and environment, weaning to inert diets) or prior to expected increase in exposure to pathogenic microorganisms and parasites. In this study, we have evaluated the effect of dietary LPS derived from *Pantoea agglomerans* (LPSp, SOMACY SL100, MACROPHI Inc., Japan) in *Anguilla anguilla* elvers considering the general condition, growth and immune status of the animals.

Elvers ( $1.7 \pm 0.2$  g) were fed at different doses of LPSp (0, 20 and 40  $\mu$ g LPSp/Kg BW/day) during 70 days at 21 °C (4 replicates per diet). LPSp was added to the diet (52% protein, 24% fat, 9% ash) by top coating with the lyophilized powder of LPSp dissolved in 2% fish oil. At the end of the trial, fish were measured for growth (BW), size dispersion, histological organization of the intestinal mucosa and the transcriptomic responses of the spleen were evaluated by means of a 60K (15,000 gene signatures) custom oligonucleotide microarray (Agilent Technologies Inc.).

No significant differences in final BW ( $3.9 \pm 0.3$  g) nor survival (98-99%) were observed among groups regardless of the dose of LPSp. The inclusion of LPSp increased goblet cell density in the intestinal mucosa in comparison to the control diet, whereas it did not affect the activity of digestive enzymes. Micorarray analysis (LIMMA  $F < 0.05$ ; adj. p value  $< 0.005$ ) resulted in 81 significantly up-regulated genes and only 3 down-regulated genes, in the highest LPSp dose. The lowest dose of LPSp up-regulated 26 genes and down-regulated 50. Both doses resulted in regulation of immune related genes, such as, sensors for detection of viral and bacterial products, innate antiviral response genes, cytokines, adipocytokines, and their regulatory nuclear factors. The pathway enrichment analysis (FatiGO;  $P < 0.005$ ) of up-regulated genes (40  $\mu$ g LPSp/kg BW/day) indicated among the most significantly regulated KEGG pathways: cytokine-cytokine receptor interaction, Jak-STAT signalling, natural killer cell mediated cytotoxicity, Toll-like, RIG-I-like and NOD-like receptor signalling and Wnt, Notch and mTOR signalling. In conclusion, LPSp diet highly regulates spleen transcription levels, mainly enhancing the immune response capacity of the immunostimulated elvers without decreasing their growth performance.

This study was funded by the CENIT-ACUISOST project (CDTI, Spain), IRTA, Basque Government (S-PE09UN32; IT810-13) and UPV/EHU (UFI 11/37).

## ASSESSING THE INCLUSION OF *Scenedesmus obliquus* IN RAINBOW TROUT (*Oncorhynchus mykiss*) DIETS: EFFECTS ON GROWTH PERFORMANCE, PROXIMATE COMPOSITION, MICROBIOTA, SERUM NON-SPECIFIC IMMUNE PARAMETERS AND FILLET COLOUR

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Microalgae meal as a protein source has been assessed in numerous studies, and no evidence indicating negative effects on growth or fish development has been reported so far. In this study authors have tested the inclusion of the green microalgae *Scenedesmus obliquus* (SCE).

Rainbow trouts (BW<sub>i</sub> = 75.5 g) were fed a diet that incorporated SCR at 5% and a control diet (48% proteins, 22% lipids). SCE (49.4% proteins, 28% lipids, 7.1% ash) was added into the diet at the expense of fish meal and oil. Diets (feed ratio = 1.5% stocked biomass) were tested for 45 days in quadruplicate at 17.7 °C when all fish were sampled for measuring their growth, fillet proximate composition and fatty acid profile, activity of antioxidative stress enzymes, gut and liver histological organization, fillet colour (Chroma Meter CR-300, Minolta Camera Co. Ltd.), microbiota analysis (16S PCR-RFLP) and serum non-specific immune response parameters.

The inclusion SCE did not affect fish growth (BW<sub>f</sub> = 154.2 – 156.9 g), nor their HSI (1.2%) or PVFI (5.7–6.1%). Fillet proximate composition was not affected by the diet, whereas SCE altered the fatty acid profile by reducing the amount of mono-unsaturated fatty acids (18:1n-9, 20:1n-9) and increasing DHA (22:6n-3) and total n-3 PUFA levels by 5%. These results may be attributed to the high content of DHA in SCE. The inclusion of *Scenedesmus* sp. did not affect the activity of oxidative stress enzymes nor the levels of lipid peroxidation in comparison to the control diet. No changes in the serum non-specific immune markers were observed between trouts fed the control and SCE containing diets, indicating that at the inclusion level tested, SCE did not have an immunostimulatory effect in the organism. SCE modified the gut microbiota composition, showing a more homogeneous gut bacterial community, whereas in terms of the histological organization of the gut, fish fed the SCE diet showed hypertrophy and higher number of intestinal goblet cells with regard to the control diet. In addition, no histological alterations were found in the liver of trouts fed both diets. Due to the high content of lutein in the microalga, the fillets of trouts fed the SCE diet had lower b\*, C\* and h\* values than fish fed the control diet. A yellowish tone noted in the fillet in trouts fed the SCE diet was also found in the serum of these animals. Present results showed no evidence of negative effects on rainbow trout condition when diets included SCE, whereas the yellowish tone of the fillet provided by SCE may be considered as a differential trait in terms of new trout products and/or marketing purposes.

This study was funded by the CENIT-ACUISOST project (CDTI, Spain) and IRTA.

## REDUCED MORTALITY OBSERVED IN EUROPEAN SEA BASS (*Dicentrarchus labrax*) EXPOSED TO *Vibrio pelagius* FED DIETS CONTAINING SHRIMP PROTEIN HYDROLYSATES (SPH)

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It is generally accepted that fish meal (FM) and oil (FO) can be partially or totally replaced in aquafeeds. However, not all species necessarily respond equally to these dietary replacements and although some generalizations may be used as a benchmark, effects of alternative protein sources should be evaluated on a case by case basis, especially with regard to the effect of FM and FO replacement on the immune response in fish. In this context, some studies have reported that the complete replacement of FM and FO by plant ingredients may negatively affect the immune response, consequently, such high plant-based diets should be balanced with health ingredients to maintain fish performance and be sustainable. We present data on the effects of the inclusion of SPH in diets for sea bass and their response to an outbreak of the pathogenic bacteria *Vibrio pelagius*, a species previously described as producer of haemolysin, a virulence factor.

In a 110 day-nutritional trial (20 °C), sea bass were fed four diets (45% CP, 16% CL, 5.1 kcal/g GE) containing different levels of FM (5, 15 and 20%) and supplemented with SPH (AQUATIV- DIANA, Symrise AG). Diets FM20, FM5 (no SPH added), FM5+SPH5 (5% SPH), FM15+SPH5 (5% SPH). FM20 and FM5 diets were used as positive and negative controls. Diets were tested with 4 replicates (450 L tanks, 60 fish/tank, BWi = 19.8 g). Fish were sampled for analytical purposes at the end of the trial. Then, all fish were collected from tanks, anesthetized and their BW measured. Then, all fish from the same dietary treatment (n = 240) were pooled together in a tank (450 L) and redistributed again into 4 new tanks. Sorting and handling activities were conducted at 21-22 °C and lasted for 1-1.5 h per dietary group.

Three days after fish handling and redistribution into the new tanks, mortalities started to occur in all tanks, with fish showing damaged fins with redness about the base of fins and mouth, and in some cases lesions of the skin. Biopsy samples from skin, kidney and tank water were inoculated on TSA, TCBS, marine broth, and blood agar plates. Colonies isolated were analysed by 16S rDNA sequencing. From BLAST analysis of the 16S rDNA it was determined the etiologic agent was *Vibrio pelagius*. Interestingly, mortality patterns significantly differed depending on the diet administered during the nutritional trial. In particular, survival rates in fish fed FM20 and FM5 were  $32.0 \pm 13.5\%$  and  $32.0 \pm 6.7\%$ , respectively, whereas those diets including the SPH showed higher survival rates (FM5+SPH5 =  $61.8 \pm 16.3\%$ ; FM15+SPH5 =  $95.4 \pm 5\%$ ). These results were not correlated to differences in growth performance, since there were no statistical differences in terms of final BW among groups (58.6 – 61.0 g). However, serum immune parameters (lysozyme, complement and bactericidal activities) measured in healthy animal samples measured before the pathogenic outbreak supported the differential mortality observed. Thus, authors conclude that SPH is a good promoter of the immune function in aquafeeds.

## DIGESTIVE CAPACITY OF MEAGRE *Argyrosomus regius*: DISTRIBUTION OF PROTEASE, AMYLASE AND LIPASE ACTIVITIES IN THE DIGESTIVE TRACT

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Meagre is the most recent species whose intensive culture has been developed in the Mediterranean basin. As a new emerging species, little information is available on the dietary requirements of meagre as well as in its digestive physiology, information that is critical for the proper development and optimization of on-growing sustainable feeds for this species.

Fish were grown for 7 months in RAS under optimal rearing conditions: 20 °C, 6.4 ppm O<sub>2</sub>, 35 ppt, 5-10% seawater daily exchange, fed with a commercial diet (EFICO PLUS 805, BIOMAR, 44CP, 18 CL; ration: 3%). Fish (162 ± 38 g, n = 30) fasted for 24 h were euthanized and their digestive tract dissected to separate the esophagus (E), stomach (S), hepatopancreas (HP), pyloric caeca (PC), anterior (IA), medium (MI), and posterior intestine (PI). Enzyme extracts from each region were prepared for measuring protein levels, pepsin-like (PEP), total alkaline protease (TAP), trypsin (T), chymotrypsin (CHYM), amylase (AMY), and lipase (L) activities.

PEP (21.1 U) activity was mainly found in the S, but a residual enzyme remained even in the PI. TAP (6.8 U) was found in the HP, PC and AI and T activity (0.4 U) in the HP and PC in contrast to CHYM activity (2.1 U) that was mainly found in the PI. AMY activity (21.6 U) was mainly found in the HP, and L (46.6 U) in the HP and CP. Digestive enzyme activities expressed as % of total activity in the digestive tract are presented in Table 1. The highest specific activity (U/mg protein) for the measured enzymes was: PEP (S) = 0.36±0.002, TAP (HP) = 0.051±0.001, T (IA) = 0.013±0.0003, CHYM (PI) = 0.082±0.001, AMY (HP) = 0.096±0.01 and L (AI) = 0.265±0.02. The S was where the PEP activity was found, but this acid enzyme was stable along the intestine, but because of the intestinal alkaline pH it should not be active. HP alkaline protease (TAP, T, CHYM) should be regarded as a proteolytic reserve, in contrast to their activities found in the CP and intestine. Proteases were 75% acid and 25% alkaline. HP AMY should be considered as a amylolytic reserve, 10% of total activity was found in the CP and intestine. HP L activity was seen as a lipolytic reserve; in contrast, 50% of total L was found in the CP and intestine. CHYM was 5 times higher than T. Lipolytic activity for releasing fatty acids (FA) of 8 C was 155 times higher than for releasing 14 C FA.

|    | Pepsin like        | Alkaline protease  | Trypsin            | Chymotrypsin       | Amylase             | Lipase             |
|----|--------------------|--------------------|--------------------|--------------------|---------------------|--------------------|
| E  | 17.8 ± 0.17        | 1.0 ± 0.13         | 3.3 ± 0.33         | 0.3 ± 0.10         | 1.1 ± 0.31          | 0.9 ± 0.07         |
| S  | <b>59.8 ± 0.30</b> | 3.5 ± 0.62         | 7.5 ± 0.43         | 0.5 ± 0.17         | 1.3 ± 0.90          | 2.4 ± 0.04         |
| HP | 5.8 ± 0.58         | <b>59.4 ± 1.69</b> | <b>57.3 ± 2.20</b> | 4.2 ± 0.47         | <b>86.9 ± 12.40</b> | <b>45.4 ± 2.01</b> |
| PC | 4.80 ± 0.67        | 12.1 ± 0.90        | 15.2 ± 0.07        | 10.8 ± 0.15        | 7.1 ± 0.45          | 24.4 ± 1.14        |
| AI | 2.10 ± 0.24        | 6.5 ± 0.30         | 8.8 ± 0.22         | 4.0 ± 0.16         | 1.7 ± 0.24          | 14.0 ± 0.7         |
| MI | 0.8 ± 0.45         | 2.4 ± 0.14         | 4.7 ± 0.97         | 1.5 ± 0.06         | 1.2 ± 0.14          | 7.4 ± 0.28         |
| PI | 8.9 ± 0.36         | 15.1 ± 0.24        | 3.1 ± 0.27         | <b>78.7 ± 1.53</b> | 0.7 ± 0.11          | 5.5 ± 0.28         |

This study received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY).

## MOLECULAR INSIGHTS OF TWO ANTI-VIRAL HOMOLOGS FROM ROCK BREAM (*Oplegnathus fasciatus*)

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Interferons are a cytokines that can be made and released by host cells in response to the presence of viruses. Many viruses trigger the type I interferon (IFN) system, leading to the transcription of hundreds of interferon-stimulated genes. IFN effectors vary widely in their magnitude of inhibitory activity and display combinatorial antiviral properties. Interferon regulatory factor 1 (IRF1) has been shown to play roles in the immune response, and regulating apoptosis. On the other hand gamma inducible lysosomal thiol reductase (GILT) plays central role in MHC class II antigen processing and presentation pathways. Putative protein sequences of RbIRF1 and RbGILT were used to predict the characteristic features. Immune responses were examined after immunization with poly I:C and rock bream irido virus (RBIV). The *RbIRF1* cDNA possessed a 314 aa peptide (36 kDa). The IRF domain and fibronectin type 3 domains was identified. Meanwhile, *RbGILT* cDNA consisted 255 aa peptide (26 kDa). Its mature peptide contained GILT signature sequence and high number of cysteine residues. Phylogenetic results showed that RbIRF-1 and RbGILT were cladded within teleostean counterpart. In addition, tissue specific mRNA expressional results have shown ubiquitous expression in 11 different tissues. Moreover, *RbIRF1* transcripts were significantly upregulated in which early post infection of poly I:C injected blood, gill, spleen and head kidney tissues. Beside, *RbGILT* significantly transcribed in gill tissues after immune challenged with poly I:C and in blood and head kidney tissues with RBIV challenge. *RbIRF1* and *RbGILT* are related to innate immune responses against viral infection and this activation may essential for subsequent adaptive immunity in rock bream.

## **RESEARCH AND TECHNOLOGY INNOVATIONS IN MOBILE PHONE-BASED FISH INFORMATION SYSTEMS IN GHANA, KENYA, AND UGANDA**

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In Africa, mobile phones are widely available and facilitate rapid transfer of information among individuals. These technologies have great potential to help improve market asymmetries between buyers and sellers of fish products and improve the bargaining power of smallholder fish farmers. As a mechanism to advance information transfer on aquaculture production, marketing, and sales, the AquaFish Innovation Lab is developing mobile-based support systems in Kenya, Ghana, and Uganda.

In Kenya, AquaFish researchers evaluated the feasibility of building a farmed fish marketing database into an existing network, called the Enhanced Fish Marketing Information System, in order to provide market information to key players in the capture fisheries sector. Researchers conducted a pilot study and a workshop to train fish farmers on the system, and determined that creating a system for synthesizing daily market information on farmed fish to end-users would bolster the aquaculture sector in Kenya.

In Ghana, researchers developed the Seafood Market Information System, a web-based tool that provides tilapia market information on-line as well as via voice and SMS/text messaging to users. AquaFish researchers worked in partnership with a local, private technology company to create customized algorithms and program an electronic information system where fisheries data and information collected from supply and demand centers is housed and transmitted to end users. This technology is now expanding to include marine artisanal fisheries and to enable accurate and real-time market price data.

In Uganda, researchers conducted focus group interviews to determine patterns of mobile phone use by fish farmers. Results indicate that mobile phones are mainly used to access technical guidance from intermediary farmers, obtain market information, accomplish mobile banking, and coordinate procurement of fish farming inputs. Results also indicate that information on stocking and feeding rates, pond management, market access and prices are most useful to farmers. In response to these findings, AquaFish researchers are working with a local technology business to develop and implement a mobile phone-based application that will enable fish farmers to access fish production and market information.



## SENSING AND TRANSPORT OF PEPTIDES IN THE GASTRO-INTESTINAL TRACT OF ATLANTIC SALMON

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Proteins are indispensable for life, making the fine control of protein intake crucial to ensure their efficient utilization for health, growth, and ultimately survival. Amino acids, the building blocks of proteins, and peptides are key nutrients in teleost diet and essential for a range of metabolic pathways. Multiple sensors present in the gut sense the degradation products of dietary proteins in the intestinal lumen and mediate a range of downstream effects. The hormonal and neural signalling pathways activated by the peptide sensors can mediate diverse functions like changes in gastric emptying and intestinal transit, release of digestive enzymes, nutrient transport, and also affect the control of food intake (hunger and satiety) and metabolism.

The intestinal peptide sensors have been so far mainly explored in mammals. They include the lysophosphatidic acid receptor 5 (LPAR5), member of the superfamily of G protein-coupled receptors, and recently, the “low-affinity/high-capacity” peptide transporter 1 (PepT1). PepT1 is the most well-characterized transporter of di- and tripeptides in fish due to its major role in protein absorption, nutrition, and possibly body growth. Recently, PepT1 has also been shown to fulfill an additional role as intestinal ‘sensor’ in mammalian enteroendocrine cells, mediating the release of gut hormones, such as cholecystokinin and glucagon-like peptide 1, into the circulation.

Atlantic salmon is a key commercial species in global aquaculture. Finding new good sources of high quality protein becomes more important as the global food industry grows. Therefore, understanding how dietary protein degradation products from different sources affect digestion and feed intake is key information for the aquaculture industry. We have started to investigate the peptide sensors in the gut of salmon to further characterise their potential role in feed intake regulation and growth. *In silico* analysis identified the Atlantic salmon LPA5R, the peptide transporters PepT1a and b, and PepT2, which is known to operate as a “high-affinity/low-capacity” transporter of di- and tripeptides. Tissue expression of the peptide sensors was investigated by qPCR in several tissues, including the gastrointestinal tract from stomach to hindgut. Furthermore, peptide sensors will be characterized functionally using a heterologous expression system, to assess the role of peptide sensing and transport in Atlantic salmon nutrition and growth. This will provide the first insights on the protein sensing system in salmon and lay the foundation for future design of optimized feed.

### ACKNOWLEDGEMENTS

Funded by Regional Research Fund West (RFFV; Grant No 247978) and Cargill Innovation Center.

## ASSESSMENT OF *Holothuria arguinensis*' GENETIC PATTERNS ON BREEDERS AND LARVAE FROM AQUACULTURE PRODUCTION

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Due to an increasing demand of sea cucumbers from oriental market, new target species from the Mediterranean Sea and NE Atlantic Ocean are being caught without control or under deficient management. This heavy fishery pressure has already provoked some important negative effects on wild populations. Therefore, the current increasing demand on sea cucumbers should be satisfied by aquaculture production. On this way, aquaculture biotechnology for these new target species is being developed with increasing success. In fact, reproduction on *Holothuria arguinensis* has been recently implemented, and larvae and juveniles have been successfully maintained.

Breeding programs under aquaculture conditions could produce loss of genetic diversity on progenies due to a low number of breeders and/or reduced effective number (low percentage of breeders contributing with eggs or sperm). Under this situation, it is easy to find inbreeding effects which can reduce fitness on progenies, with probable reduction on growth and survival rates and defective development of embryos and larvae.

In this work, we carry out the assessment of genetic diversity on *H. arguinensis*' breeders (caught from wild stocks in Ria Formosa, S Portugal) and progenies (*mid auricularia* and *pentactula* larvae) obtained from two reproduction events induced by increasing water temperature during summer 2015. Genetic characterization was done using DNA nuclear markers (7 microsatellites) and genetic diversity was assessed considering the allele richness (AR), private allele number (PA), observed and expected heterozygosity (Ho and He), presence null alleles (NA), Hardy-Weinberg equilibrium (HWE) and Fis. To evaluate the genetic differentiation among breeders and different larvae stages, a factorial correspondence analysis (FCA) was carried out using allele frequencies and Fst values were calculated too. These analyses were complemented with the estimation of genic and genotypic differentiation.

Breeders from the first event had higher genetic diversity (AR: 7.29; He: 0.74; PA: 11) than those from second event (AR: 3.28; He: 0.68; PA: 2). It was observed an interesting trend on both reproductive events: gradual loss of genetic diversity from breeders to *mid auricularia* and *pentactula* larvae. Fis values (=inbreeding) did not detect deficit of heterozygotes on breeders from first event, but it was found deficit on 3 loci from second event. *Mid auricularia* larvae from second event showed the highest loci number (5) with deficit of heterozygotes. This could be due to the lower number of eggs (210.000) obtained from this second event comparing with the first one (1.47 millions of eggs).

The FCA, Fst values and genic and genotypic analysis found significant differences between *mid auricularia* from the second event, breeders and the rest of larvae belonging to the two events. These results are indicating that *mid auricularia* from the second event (with low number of eggs) are showing a genetic pool different to the surviving *pentactula* larvae: a lower number of eggs could be harboring a low percentage of "optimal genotypes" for survival. In fact, most of *pentactula* from the second event are similar (genetically) to the *mid auricularia* and *pentactula* larvae from the first event, where 1.47 millions of eggs were obtained. Adults from both events were different to larvae, which could imply selection processes with loss of genetic diversity.

## **EMPOWERING WOMEN AND YOUTH CAN ADVANCE SUSTAINABLE AQUACULTURE AND INCREASE FOOD SECURITY**

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Empowering women and youth with knowledge and skills on aquaculture can positively impact household nutrition, food security, and income. However, women's potential to contribute to sustainable aquaculture and improved nutrition is limited by restricted market opportunities, lack of access to education, and inequalities in economic participation and decision-making power. Likewise, Africa's youth ( $\leq 25$  years), comprising over 50 percent of the continent's population, encounter similar obstacles. Not only are women and youth tied to each other by their barriers to participation in the agriculture sector, they are also intrinsically linked by maternal health through early childhood development.

Mothers, as gatekeepers of household nutrition, have the power to improve children's health — if given the opportunity. Undernutrition in the first two years of a child's life can have permanent consequences on their well-being. Between 50 and 70 percent of youth in Africa rely on agriculture for food and employment, while over 40 percent of rural workers are women; however, only five percent of women receive extension services.

Towards uplifting these marginalized groups, the Feed the Future Innovation Lab for Collaborative Research on Aquaculture & Fisheries (AquaFish) conducts research and training activities that engage women and youth on sustainable aquaculture practices. Since 2006, AquaFish has engaged over 3,300 women and girls in short-term trainings on sustainable aquaculture and household nutrition. In 2016, 50 percent of degree-seeking students supported by AquaFish were women.

## THE USE OF DIETARY INGREDIENTS DERIVED FROM FISH PROCESSING BY-PRODUCTS IN ABALONE DIETS, WITH AND WITHOUT FORMIC ACID SUPPLEMENTATION

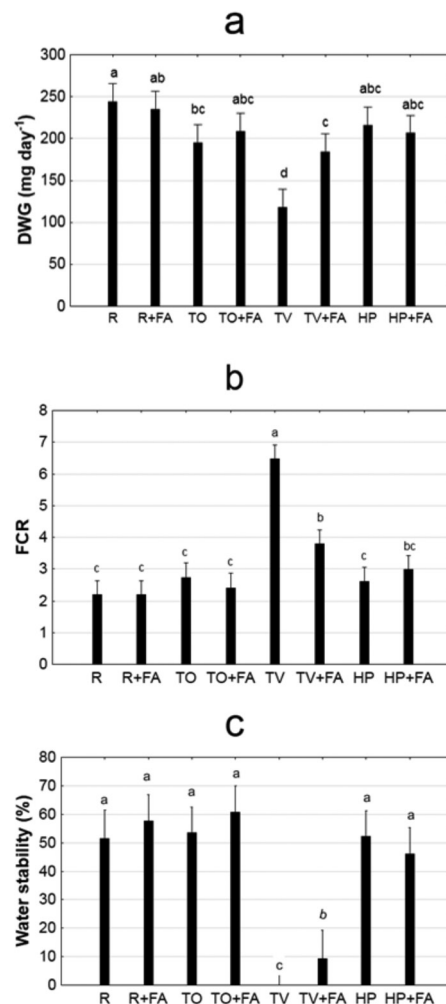
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The use of ingredients derived from by-products is becoming increasingly common aquaculture feeds, due to their good nutrient profiles e.g. high content of essential amino acids and fatty acids. In some instances, by-products are utilised by addition of organic acids to ensure microbial preservation, after which the preserved by-products are included in formulated diets. Formic acid is commonly used as such an antimicrobial preservative of wet and moist feed ingredients e.g. in fish silage; however, formic acid and its salts are known to also improve aquaculture production efficiency even when included on their own (i.e. not as part of by-product derived feed ingredients). During feeding trials with the South African abalone *Haliotis midae*, various feed ingredients derived from fish processing by-products were incorporated into formulated aquaculture diets, and the aim of this study was therefore to distinguish between possible effects of formic acid and the feed ingredients evaluated.

The study evaluated four formulated diets with and without formic acid supplementation: a commercial reference (diet R), and three diets where the commercial diet was supplemented with 1) fish oil recovered from rainbow trout silage (TO), 2) minced, unprocessed rainbow trout viscera (diet TV) and 3) a commercial hydrolysed shrimp by-product protein (HP). Growth trials were conducted on a commercial abalone farm to evaluate the diets. The trial results were evaluated at the hand of animal production performance, and the water stability of the experimental diets.

It was found that diets containing unprocessed trout viscera had significantly ( $P < 0.05$ ) lower weight gain (DWG) and a higher feed conversion ratio (FCR), respectively; however, formic acid inclusion improved DWG and decreased FCR in diets containing viscera. There were no effects of formic acid in any of the other diets. The improved production performance caused by formic acid in the viscera-containing diets, was correlated to improve feed water stability (Figure 1). The results indicate that formic acid inclusion can play a role in enhancing the water stability in aquaculture diets, and thereby improve production performance. This is of particular importance for slow-feeding aquaculture species such as abalone.



**Figure 1** Daily weight gain (DWG), feed conversion (FCR) and water stability of the different treatments.

## **ABALONE (*Haliotis midae*) BEHAVIOUR AND STOCKING DENSITY: IS BEHAVIOUR INFLUENCED BY DENSITY?**

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The profitability of abalone farms is influenced by production per unit grow-out space. Production is a function of abalone growth and stocking density, yet little is known about abalone behaviour. The aim of this study was to gain a better understanding of the behaviour of South African abalone, *Haliotis midae*. An objective of the study was to characterise abalone behaviour and compare behaviour at a low (18 % of the available surface area, SA) and high (25.5% SA) stocking density.

This objective was quantified using focal animal follow protocols and quadrat sampling through a clear-sided PVC tank. There appears to be a tradeoff between the percentage of abalone locomoting (L) and grazing (G). The ratio of abalone L(%):G(%) was significantly higher at HD (74:18) when compared to LD (49:42). When comparing the proportion of time abalone spent locomoting at night, abalone at HD spent on average 18% more time locomoting when compared to LD (Ttest,  $t_{46} = 2.67$ ,  $p = 0.01$ ).

Results demonstrate that abalone are more active (i.e. expend more energy moving around the basket) at a higher density and that they spend less time grazing on diatoms at higher densities than at lower densities. These factors in combination have the ability to influence the growth rate of abalone.

## QUANTIFICATION DE LA DÉRIVE GÉNÉRÉE PAR L'UTILISATION DES DEUX TYPES DE PULVÉRISATEURS LORS DES PULVÉRISATIONS SUR LE COTONNIER

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L'objectif global de cette thèse est d'initier pour évaluer les risques de contamination lors des traitements phytosanitaires, réalisés selon les pratiques qui sont observées localement avec les pesticides en culture cotonnière, effectués au moyen de la canne centrifuge ou du pulvérisateur à dos. A cet effet, une enquête semi a été réalisée auprès de 115 producteurs de coton sur un total de 172 producteurs que compte la coopérative villageoise des producteurs de coton de Batran à Banikoara, afin de se rendre compte de l'état des lieux du respect des bonnes pratiques phytosanitaires en production cotonnière. Ensuite, 10 essais de pulvérisation de bouillie de tartrazine, à la canne de pulvérisation centrifuge et au pulvérisateur à dos, à des hauteurs de pulvérisation de 1 m et de 1,5 m, ont été réalisés en champs de coton par différents producteurs de coton, pour évaluer le risque d'exposition des opérateurs lors des traitements phytosanitaires. Il en a été de même, mais sur des sols nus, pour l'appréciation de la dérive de pulvérisation lors des traitements phytosanitaires. La méthode des patchs a été utilisée pour la contamination du corps de l'opérateur tandis que des collecteurs ont été placés à des altitudes déterminées à des distances régulières par rapport à la zone de pulvérisation pour recueillir la dérive de pulvérisation. L'enquête a révélé que les bonnes pratiques phytosanitaires ne sont pas bien respectées par les producteurs de coton de Batran. Les mesures de masses de tartrazine recueillies sur différentes régions du corps de l'opérateur, ont révélé d'une part, que les cuisses sont les parties les plus exposées et d'autre part, que la hauteur de pulvérisation de 1,5 m contamine plus le corps de l'opérateur que celle de 1 m. De plus, la canne de pulvérisation centrifuge contamine plus le corps de l'opérateur que le pulvérisateur à dos. L'évaluation de la dérive de pulvérisation a montré que cette dernière suit une distribution gaussienne. Mieux, la hauteur de pulvérisation de 1,5 m et la canne de pulvérisation centrifuge conduisent à des dérives plus nuisibles que respectivement la hauteur de pulvérisation de 1 m et le pulvérisateur à dos.

## CONTAMINATION DES ECOSYSTEMES AQUATIQUES PAR LA DERIVE DES PRODUITS PHYTOSANITAIRES

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L'objectif global de cet article est d'analyser les voies de transfert des pesticides vers écosystèmes aquatiques lors des traitements phytosanitaires. A cet effet, une enquête semi a été réalisée sur les pratiques phytosanitaires auprès de 115 producteurs de coton sur un total de 172 producteurs que compte la coopérative villageoise des producteurs de coton de Batran à Banikoara. Ensuite, 10 essais de pulvérisation de bouillie de tartrazine, à la canne de pulvérisation centrifuge et au pulvérisateur à dos, à des hauteurs de pulvérisation de 1 m et de 1,5 m, ont été réalisés en champs de coton par différents producteurs de coton, pour évaluer la distance à laquelle les gouttelettes de pesticides dérivent en tenant compte des paramètres climatiques. Aussi, des prélèvements de sols ont eu lieu pour déterminer l'impact de la nature du sol sur le transfert via le ruissellement.

Il ressort des résultats de notre étude que le flux des contaminants agricoles vers les eaux de surface résulte de l'interaction complexe entre les activités agricoles, la nature des sols, les conditions climatiques et la structure du bassin versant auxquelles s'ajoute l'organisation du paysage. Le ruissellement est un des facteurs favorisant le transfert des produits phytosanitaires vers les retenues d'eau. De forte pente du relief qui converge vers les retenues d'eau favorise également le transfert. Dans ce contexte, lorsque les produits phytosanitaires sont utilisés à des doses supérieures à celles recommandées, sous l'effet d'une forte pluie le surplus retenu par le sol ou les feuilles des plantes se retrouve facilement dans les eaux de surface grâce aux ruissellements des eaux.



## ANALYSE PAR LA METHODE DPSIR DES RISQUES ENVIRONNEMENTAUX LIES AUX PRATIQUES PHYTOSANITAIRES EN CULTURE COTONNIERE AU BENIN

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Au Bénin, les différents écosystèmes (milieux terrestres et aquatiques), sont caractérisés par une présence généralisée de résidus de pesticides liée à l'utilisation sans cesse croissante et incontrôlée de ces produits dans l'agriculture notamment sur la culture cotonnière. Cette pratique phytosanitaire même si elle permet de lutter efficacement contre les ravageurs engendre des risques pour l'environnement et la santé humaine. L'objectif de cette recherche est d'évaluer les risques sanitaires et environnementaux liés aux pratiques phytosanitaires en production cotonnière au Bénin et d'évaluer l'efficacité des actions menées pour minimiser ces risques. A cet effet, une analyse macroéconomique de la filière coton a été faite à travers l'approche DPSIR (*Driver-Pressure-State-Impact-Response*) basée sur les liens de causalité entre les composantes des systèmes culturels et l'environnement. Il ressort de cette analyse que les forces motrices qui incitent à l'utilisation des pesticides dans la filière coton au Bénin se résument à sa contribution au PIB (13%), sa création d'emploi et de revenu monétaire, les politiques de soutien (crédit intrant, crédit aux cotonculteurs), la résistance des ravageurs et l'absence de sanction suite aux mauvaises pratiques phytosanitaires.

Ces forces motrices produisent des pressions sur l'environnement qui se traduisent par l'évolution des quantités de pesticides utilisés, qui dès lors dégradent l'état de l'environnement avec des impacts sur la santé humaine et la biodiversité des écosystèmes. Ces impacts ont conduit la prise de diverses mesures politiques dont la ratification des conventions / accords, la création des structures de réglementation de la pollution et des textes juridiques sur les produits phytosanitaires au Bénin qui peine à être effective dans la mise en œuvre.

## EFFECT OF DIETARY PROTEIN ON HYBRID TILAPIA PRODUCTION IN A BIOFLOC TECHNOLOGY SYSTEM

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In an outdoor biofloc technology (BFT) production system a complex of living organisms that includes phytoplankton, zooplankton, ciliates, nematodes, bacteria, all closely associated with particulate organic matter, is maintained suspended in the water column by continuous aeration. Tilapia grown in the BFT system can derive nutrition by consuming the biofloc, and because of this some researchers advocate reducing formulated ration protein content. Using the ratios and concentrations of essential amino acids in hybrid tilapia muscle, i.e., the ideal protein model we selected, as targets for optimizing diet formulation, we formulated three diets based on digestible nutrient content of feed ingredients and supplemented the formula with essential amino acids to meet the target levels suggested by the muscle profile. Diets were formulated to contain 22.5%, 27.7%, and 32.3% intact digestible protein and 6% lipid.

Hybrid tilapia (*Oreochromis aureus*  $\square$  x *O. niloticus*  $\square$ ; 32.2 g/fish) were stocked at 25 fish/m<sup>2</sup> into each of nine outdoor 18.6-m<sup>2</sup> BFT system production tanks and grown for 146 d. Fish were fed their respective diets to apparent satiation twice daily.

Fish consumed each diet equally well. Feed consumption during the peak feeding period (28 Jun-8 Oct) and the entire experiment did not differ significantly and averaged 194.4 g/m<sup>3</sup>/d and 22.2 kg/m<sup>3</sup>, respectively. Production varied among diets, with significant differences between the 22.5% and 27.7% protein diets, with the 32.3% protein diet being intermediate. Mean final weights were 517.9, 564.5, and 552.6 g/fish for the low to high protein level, respectively. Respective gross fish yields were 14.0, 15.6, and 14.9 kg/m<sup>3</sup>. Feed conversion ratio (FCR) was significantly lower for the 22.5% protein diet (1.5) compared to an FCR of 1.3 for the other two diets. Survival did not differ significantly and averaged 98.8%. Hepatosomatic index decreased linearly and muscle ratio increased linearly in response to increasing dietary protein. Intraperitoneal fat did not differ significantly among dietary protein. Geosmin and 2-methylisoborneol (MIB) were detected in fillets sampled at harvest from fish from each treatment tank. Only one replicate tank each from the 22.5% and 32.3% protein treatments contained fish with geosmin and MIB concentrations that likely would be designated as being “on-flavor” when evaluated by trained processing plant flavor testers. The source of the off-flavor remains to be determined. Results of this experiment suggest that diet protein can be reduced by 5% to 27.7% digestible protein with no adverse effect on production, but further reduction causes a loss in productivity, but an economic evaluation is needed.

## DEGREE-DAYS AS A TOOL FOR USE IN PRODUCING TILAPIA FRY FOR SEX INVERSION

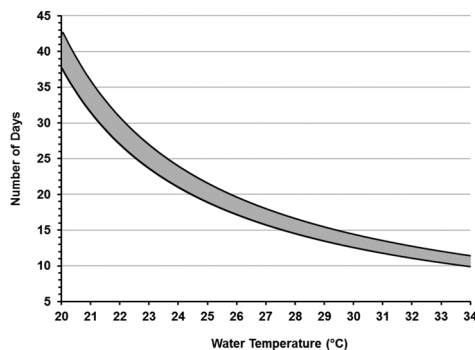
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Hormonal sex inversion of newly hatched tilapia fry continues to be an important method to produce monosex male tilapia fingerlings. Large numbers of tilapia fry suitable for sex inversion can be produced by periodic complete harvest of earthen reproduction ponds. Traditionally, harvest interval was based on the assumed tilapia reproductive cycle. Water temperature affects tilapia reproduction and growth, and in the tropics and subtropics varies seasonally and with elevation. Degree-days, also known as the rule of thermal summation, is a calculation used to quantify temperature effects on biological processes, and has been used by entomologists, agronomists, and, to a lesser extent, fisheries biologists. The relationship between degree-days and production in earthen ponds of fry suitable for sex inversion was quantified in the tropics for Nile (*Oreochromis niloticus*) tilapia and in the subtropics for Nile and blue (*O. aureus*) tilapia.

Earthen reproduction ponds (0.01-0.05 ha) in Honduras (tropics) and Egypt (subtropics) were stocked with tilapia broodfish at 0.6-1.0 fish/m<sup>2</sup> (1.5-2.2 females per male). Ponds were drained 13-21 days after stocking, the broodfish removed en masse and transferred to a holding tank, and fry harvested from the harvest sump using dipnets (1.6-mm ace nylon mesh). Harvested fry were graded through 3.2-mm square mesh plastic netting and numbers determined; fry passing through the netting were suitable for sex inversion whereas retained fry were too large. A total of 33 and 26 independent trials were conducted in Honduras and Egypt, respectively. Degree-day was calculated by subtracting the base temperature (15°C) from the mean hourly water column temperature and summing over the duration of each trial.

No tilapia fry were observed at fewer than 140 degree-days in Honduras, although females incubating eggs were observed at 120-140 degree-days, whereas in Egypt no fry were observed at fewer than 125 degree-days. Total fry production in both countries increased linearly as degree-days increased from 125-276, but the proportion of fry too large for sex inversion increased linearly as degree-days increased beyond 200. Total length of fry suitable for sex inversion averaged 9.4 and 8.9 mm for Nile and blue tilapia, respectively, and 14.4 mm for fry too large for sex inversion. Range in number of days until reproduction pond harvest in relation to mean weekly pond water column temperature for optimal production of Nile tilapia and blue tilapia fry (7-13 mm total length) suitable for hormonal sex inversion. Optimal production of fry suitable for sex inversion occurs between 190 degree days (lower curve) and 220 degree days (upper curve).



## CHANNEL CATFISH PRODUCTION IN A BIOFLOC TECHNOLOGY SYSTEM

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High yields are obtained from an outdoor biofloc technology (BFT) production system in response to high stocking and feeding rates because the biofloc, which is maintained in suspension by continuous aeration, metabolizes excreted feed nitrogen (N). Penaeid shrimp and tilapia are grown in the BFT system because they can derive some nutrition from consuming the biofloc. For fish like the channel catfish (*Ictalurus punctatus*), which derives little or no nutrition from the biofloc, the BFT system is a water quality management tool that permits production intensification and increased water use efficiency. We investigated channel catfish production, water quality and phytoplankton dynamics, and common off-flavors in a series of production experiments conducted in outdoor, 18.6-m<sup>2</sup> tanks.

Effect of stocking rate on fingerling to stocker (115-150 g/fish) production was evaluated. Survival averaged 95.1%. Fingerlings (48 g/fish) were stocked at 26, 35, 44 fish/m<sup>2</sup> and grown for 183 days. Gross fish yield (GFY) increased linearly from 4.9 to 7.1 kg/m<sup>3</sup> as stocking rate increased. Final weight (FW) averaged 172.6, 150.8, and 145.5 g/fish for the low to high stocking rates, and did not differ significantly. Growth of stocker catfish (217 g/fish) to market size was evaluated at stocking rates of 5.4, 8.1, and 10.8 fish/m<sup>2</sup> in a 154-d study. Mean survival was 97.2%. GFY increased linearly from 5.2 to 8.2 kg/m<sup>3</sup>, but mean FW decreased linearly from 828.9 to 658.4 g/fish in response to increasing stocking rate. Fingerlings (49 g/fish; 12 fish/m<sup>2</sup>) were grown to market size in tanks subjected to three solids management protocols. Settling chamber influent flow rates were 0, 0.6, and 2.5 L/min. Solids removal significantly reduced total suspended solids from 662 to 290 mg/L, but did not affect fish production. Mean GFY and FW were 8.0 kg/m<sup>3</sup> and 571 g/fish, respectively.

Operation of the BFT system during the cold season showed that catfish can be held with minimal weight loss, and in absence of sustained ammonia-nitrogen input that the biofloc retained its ability to biotransform ammonia regardless of whether phytoplankton or suspended solids predominated. An active biofloc at the beginning of the growing season obviates the start-up time to establish a new, fully functional biofloc and associated ammonia and nitrite spikes.

Fast-growing, unicellular and small colonial types of green algae, diatoms, and slower growing, small colonial types of cyanobacteria dominated phytoplankton communities. BFT culture tanks were susceptible to episodes of geosmin and 2-methylisoborneol (MIB) in the tank waters and subsequent bioaccumulation of these compounds in the catfish flesh. While levels of geosmin and MIB in the tank waters were less intense and less persistent than episodes that can occur in catfish aquaculture ponds, the microbial sources responsible for geosmin and MIB in BFT tanks remains unknown and requires additional research.

## “SISTEMINHA EMBRAPA” — AN INTEGRATED SYSTEM FOR FOOD PRODUCTION

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The “Sisteminha Embrapa”, an integrated system of food production, is a tool to combat hunger and reduce poverty. The system can be established on land sizes of as low as 100 m<sup>2</sup>. Raised fishponds of 8000 L capacity, built with various materials such as cardboard and plastic, as the main module is used in fish rearing. Fish (tilapia) can reach weights of 150-200g in 90 days with production of up to 30 kg. Bacteria are created in the biological filter, allowing the reduction of toxic ammonia in nitrite and nitrate, harmless to fish and viable to plants. Besides the fish rearing, other food production units including poultry, guinea pigs, goat, pig, insects and earthworms are integrated in the system (Figure 1). The biodigester for the production of gas and/or composting is also part of the waste recovery process. Composting and vermiculture provide N, P, K, Ca and Mg for vegetables such as corn, beans, squash, sweet potatoes, cassava, yams, okra, tomatoes, gherkins, papaya, watermelon, etc. which are also produced. The 14 production modules are aggregated according to the will of the family members, who decide what to produce. In this model of family production, there is no initial commitment of production to the market; all products are used for the family’s upkeep. All production is staggered, which allows the consumption without interruption, all year round. The diversity of products guarantees the sustainability of the business. Generally there is a surplus of products that may or may not stimulate family entrepreneurship. If so, the money raised from sales is spent on the construction of new modules and the purchase of inputs, while maintaining the sustainability of the system. Over 2500 units are scattered throughout Brazil. In Africa, Ghana, Uganda, Tanzania, Cameroon, Ethiopia and Mozambique stand out as current multipliers of technology.



Figure 1. Fish tank, farmed tilapia; vegetable crop and poultry integrated to fish farming.

## METABOLIC DIFFERENCES TO TWO POPULATIONS OF THE MUD CRAB *S. paramamosain* IN CHINA

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Mud crabs are distributed only along the coastal areas of southeast China in the south of Yangtze River Estuary. Four species of mud crabs found are *Scylla serrata*, *S. tranquebarica*, *S. paramamosain*, *S. olivacea*. *S. paramamosain* is the dominant species and distributes along all the coastal area of southeast China, while the other three species only distributes along the coastal areas of southern China around Hainan Island and the coastal area of Beibu Gulf. Qiongzhou strait blocked their gene flow and caused genetic differentiation among the strains of Beibu Gulf and that of mainland China. The genetic diversity of *S. paramamosain* in China is decreased geographically from south to north. There are morphological differences in different geographical samples mainly in the color of carapace and the number of spine on the outer margin of the cheliped carpus. Morphological diversity of *S. paramamosain* in China is also decreased geographically from south to north. The above information indicates that there are a certain genetic differentiations for *S. paramamosain* in China to northern and southern population. Research has been conducted to measure and compare the mitochondrial respiration rate and enzyme activity of northern and southern population of *S. paramamosain* in China sampled during low temperature seasons and from three locations at different latitudes. It was shown that for both northern and southern population, with the exception of lactate dehydrogenase and pyruvate kinase, the mitochondrial respiration rate and enzyme activity of crabs collected from higher latitude coasts of Ningbo were significantly higher than those from Danzhou at lower latitude. As for comparison between northern and southern population collected at a same location, the mitochondrial respiration rate and cytochrome C oxidase activity in the gill, muscle and hepatopancreas of crabs of northern population were in general higher than those of southern population from all three locations with the differences increased with the increase of latitude. Of lactate dehydrogenase, pyruvate kinase and hexokinase activities in muscle, hexokinase activities of crabs of northern population were also significantly higher than those of southern population collected from Ningbo. The results of the study indicated that northern population of *S. paramamosain* had a strong metabolic compensation capacity than southern population under cold environment, especially at higher latitude, confirming the hypothesis that *S. paramamosain* in China has differentiated into northern and southern population.

### Acknowledgements

This work was supported by a grant (31472294) from National Natural Science Foundation of China (NSFC).



# REPLACEMENT OF ANCHOVY MEAL BY GIBEL CARP (*Carassius gibelio*) MEAL IN RAINBOW TROUT (*Onchorhynchus mykiss*) DIET

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Gibel carp one of the nonnative and invasive fish species in Europe. Also gibel carp have high reproductive capacity and very fast spread in nature. Due to its features can be possible harmful on native environment. Hence, this study has been conducted to determine the replacement of anchovy meal by gibel carp meal for economic benefits of invasive species to aquaculture.

Gibel carp catches with longlines with some dam lake in Turkey and then fish meal performed with standard processes. Three diets prepared for rainbow trout juvenile, which is replaced with anchovy meal 50% and 100%. Fish were stocked 100 L aquarium (25 fish per aquarium) with free flow water system. Healthy cultured average  $4.58 \pm 0.09$  g (mean weight  $\pm$  SD) rainbow trout were stocked at 9 aquariums (100 L) with 25 fish each for triplicate study in a 60-day feeding trial.

The fish were fed twice daily mid-morning and mid-afternoon to apparent satiation with the preparing diets. Dissolved oxygen, pH, temperature and conductivity of the water were  $7.32 \pm 0.28$  mg L<sup>-1</sup>,  $7.7 \pm 0.3$ ,  $18.9 \pm 0.6$  °C and  $559.3 \pm 44.5$   $\mu$ S sec<sup>-1</sup>, respectively.

Fatty acid analyses were carried out on dorsal muscle of the rainbow trout.

At the end of trial, proximate analyses performed from fish meat on all groups.

According to this study results, anchovy meal can be replace with gibel carp meal in rainbow trout diets.

Ps: This study supported that Kastamonu University Scientific Research Foundation, which number is KÜ-BAP01/2016-26.

Table 1. Composition of the diets

|                           | <i>Ingredients (g kg<sup>-1</sup>)</i>          |       |       |
|---------------------------|---|-------|-------|
|                           | Control   | 50%   | 100%  |
| Anchovy meal              | 47.00   | 23.50 | -     |
| Gibelio meal              | -   | 23.50 | 47.00 |
| Soybean meal              | 20.00   | 20.00 | 20.00 |
| Wheat                     | 11.00   | 11.00 | 11.00 |
| Corn starch               | 7.50  | 8.00  | 8.50  |
| Vit-Min. mix <sup>a</sup> | 2.00  | 2.00  | 2.00  |
| Fish oil                  | 12.50   | 12.00 | 11.50 |
|                           | <i>Chemical composition (g kg<sup>-1</sup>)</i> |       |       |
| Protein                   | 38.40   | 38.87 | 38.93 |
| Lipid                     | 15.53   | 15.39 | 15.47 |
| Ash                       | 11.17   | 12.21 | 13.16 |
| Carbohydrate              | 32.90   | 30.45 | 28.43 |

<sup>a</sup>Vit-Min. (Vitamin-Mineral) per kg diet: 18000 IU retinyl acetate, 2500 IU cholecalciferol, 250 mg DL- $\alpha$ -tocopheryl acetate, 12 mg menadione sodium bisulphate, 0.06 mg cyanocobalamin, 200 mg ascorbyl polyphosphate, 1 mg D-biotin, 2000 mg choline chloride, 10 mg folic acid, 36 mg pantothenic acid, 50 mg pyridoxine, 50 mg riboflavin, 25 mg thiamin, 120 mg inositol, 270 mg nicotinic acid, 75.3 mg Fe, 12.2 mg Cu, 206 mg Mn, 85 mg Zn, 3 mg I, 0.350 mg Se, 1 mg Co.

Table 2. Weight gain, specific growth rate (SGR), and feed conversion rate (FCR)

| Parameters  | Control            | 50%                   | 100%               |
|-------------|--------------------|-----------------------|--------------------|
| Weight gain | $84.77 \pm 3.65^a$ | $92.76 \pm 1.18^{ab}$ | $99.30 \pm 6.40^b$ |
| FCR         | $1.62 \pm 0.06^a$  | $1.45 \pm 0.03^b$     | $1.28 \pm 0.08^c$  |
| SGR         | $1.02 \pm 0.03^a$  | $1.09 \pm 0.01^{bc}$  | $1.15 \pm 0.05^c$  |

Table 3. Fatty acid profiles of the groups

| Parameters    | Control | 50%   | 100%  |
|---------------|---------|-------|-------|
| $\Sigma$ SFA  | 22,38   | 25,08 | 22,86 |
| $\Sigma$ MUFA | 21,28   | 26,36 | 20,62 |
| $\Sigma$ PUFA | 35,76   | 36,28 | 33,83 |

Table 4. Composition of the fish meat

| Parameters | Control | 50%   | 100%  |
|------------|---------|-------|-------|
| Moisture   | 66.89   | 67.05 | 66.31 |
| Protein    | 17.45   | 18.02 | 18.65 |
| Lipid      | 14.50   | 13.96 | 14.01 |
| Ash        | 1.16    | 0.97  | 1.03  |

According to this study results, anchovy meal can be replace with gibel carp meal in rainbow trout diets.

Ps: This study supported that Kastamonu University Scientific Research Foundation, which number is KÜ-BAP01/2016-26.



## **INTEGRATING EXPERT JUDGMENT IN VETERINARY EPIDEMIOLOGY: EXAMPLE GUIDANCE FOR DISEASE FREEDOM SURVEILLANCE**

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Expert opinion can provide much needed guidance when decisions are pressing and empirical data are lacking. Expert opinion can informally support decisions regarding disease prevention and response. However, when structured for the task, expert opinion can also rapidly generate data and estimate measures of association akin to that more typically derived through field study. In contexts in which speed of assessment is critical, such as during or immediately following a disease outbreak, expert opinion elicitation can offer an extremely valuable contribution to the evolving science on the pathogen. However, opinion is clearly subjective and by nature subject to vagaries. Consequently, the manner in which opinion is gathered can impact the quality of its results. Decision science fields have a long history in methods to elicit structured and reliable judgment. Recent evaluations of animal population health have benefited from expert elicitation components. This talk will discuss key findings relevant to expert opinion elicitation for veterinary fields, and describe recent applications to animal agriculture.

## USING GIS CLASSIFICATION METHODS TO PREDICT SUITABLE HABITAT FOR SEA RANCHING OF CULTURED SANDFISH *Holothuria scabra* IN PAPUA NEW GUINEA

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Mariculture of the commercial holothurian, sandfish (*Holothuria scabra*), may assist in restoring overfished populations and generating income for coastal communities in the Pacific Islands region. Sea ranching of sandfish, where cultured juveniles are stocked into community marine tenure areas in a 'put, grow and take' activity, is currently being trialed in Papua New Guinea (PNG). The success of this approach relies on growth and retention of released cultured juveniles, which is influenced by the quality and extent of suitable habitat. Recent studies from countries farming sea cucumbers (e.g. Madagascar, Philippines, PNG) show that certain biophysical features are advantageous for sandfish mariculture. This is important but has limited application for every potential mariculture site because it relies on time-consuming and expensive field/laboratory work to obtain those data.

In an attempt to develop a useful yet cost effective method to identify suitable sandfish habitat, geographic information systems (GIS), satellite imagery and biophysical survey data were applied to trial sea ranch sites in New Ireland Province, PNG. Classified Worldview satellite imagery and field data were combined to identify different shallow water habitats, and GIS was then used to match these habitats with sandfish distribution and abundance data.

If this technique is shown to have reasonable reliability then it will be applicable to sandfish mariculture planning through prediction of viable sea ranching sites for this species. It may also have utility for (a) assessing remote areas where field survey is expensive; (b) estimating potential production for large areas; and (c) managing unrealistic community expectations in areas with suboptimal habitat.

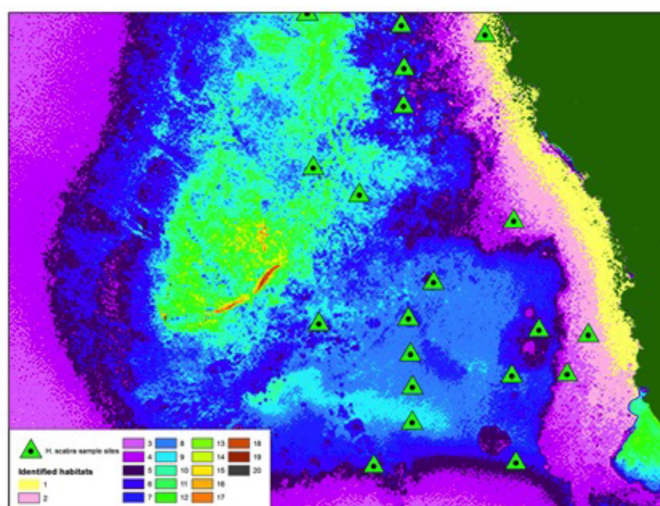


Figure 1: Shallow water classes from World view 3 imagery and *H. scabra* field sites

## CONSUMER PREFERENCES AND CONSUMPTION PATTERNS FOR FISH IN UGANDA

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The current government policy on aquaculture is promotion of the subsector to compliment the dwindling capture supplies from the wild to improve food fish, nutrition and eradication of poverty. Much as the government is pursuing this policy there exist limited information on the consumer behavior between captured and farmed fish. The objective of the study was to establish consumer preferences and consumption patterns for the two categories of fish. This study was carried out in the purposively selected districts representative of Uganda fish consuming community living near major landing sites, that is, Nebbi, Kampala, Busia, Kasese, Kisoro and Kabale . A total of 250 consumers were randomly selected and interviewed using a structured questionnaire. Descriptive statistics and regression analysis were the methods used to analyze the data.

The results show that the consumers' average age was 33years having a household size of 6.74persons and earning 628,200UGx monthly. Distance to fish source was 3.49km while 70% of the respondents had eaten fish as a protein source for an average of 23 years. 92.5% bought tilapia which was mainly (62.2%) captured fish. When buying fish, 70% of fish consumers considered fish species as the most important attribute. The majority (55%) of consumers purchased their fish from traditional markets and the rest from road side markets and landing sites. On average, consumers bought fish about 6 times per month, resulting in total consumption of 13.86 kgs. Thirty two percent of the fish consumers perceived the fish obtained from shallow muddy waters as of low quality, while 32% perceived farmed fish as more bonny and small (300gms) compared to capture fish which are fleshy and averaged a weight of 500gm and above. Many consumers (67.5%) preferred smoked fish and mainly (75%) prepared fish by boiling method.

Econometric results show that annual household income and education level significantly affected fish consumption patterns. In view of the results, it is recommended that researchers should breed fleshy easy to farm fish species which can grow to 500gm preferred by consumers. In order to address the issue of muddy fish smell, there may be a need to design fish production systems that avoid fish proximity with mud during the production process.

## EFFECT OF LIGHT, FISH BIOMASS AND NATURAL ANTIFOULING AGENTS ON OCCURRENCE AND DIVERSITY OF MARINE MACRO-FOULING IN SILVER POMPANO CAGE CULTURE

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The effects of light, fish stocked biomass and effectiveness of *sifa* and neem extracts on occurrence and diversity of marine macro-fouling in cage culture in Tanzania coastal water were investigated. A total number of 26 species and multispecies categories of fouling organisms were identified with total species richness ranging from 5 to 19 species per sampling time. The stocking biomass, sampling duration and antifouling extracts had a significantly effects on the fouling biomass and diversity. After 90 days of immersion, the antifouling efficiencies decreased significantly on both *sifa* and neem extracts. And the species reaches and percentage cover of biofouling community had reached an equal distribution on both treatments and control nets. However, dry weight of mollusca were significantly higher than that of algae and crustaceans for both treatments ( $12.6 \pm 3.2$ ,  $13.8 \pm 1.6$  and  $11.2 \pm 2.1$ ) *sifa*, neem, and control nets panels respectively. The results of this study may contribute to the understanding on the effect of cage stocking biomass and optimisation of natural extracts antifouling for treatments of aquaculture nets.

Figure 1: Biofouling species richness from three different net treatments

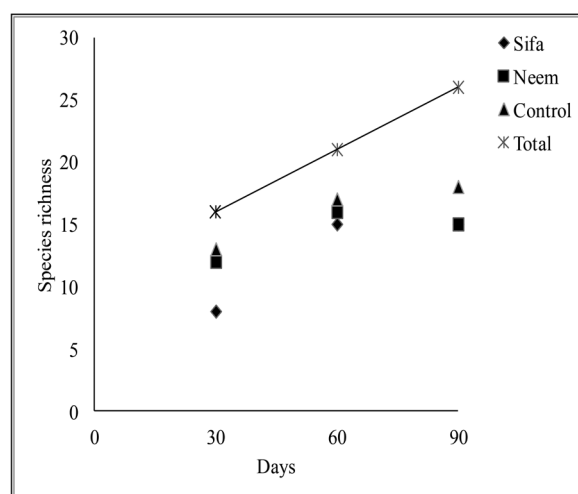
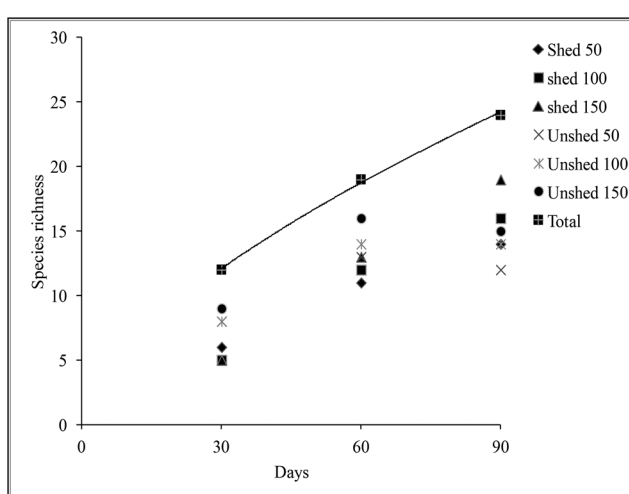


Figure 2: Species richness of biofouling from Un-shaded and shaded zones at three different stocked densities



## AQUEOUS CALYCE EXTRACT OF *Hibiscus sabdariffa* AS AN ANTI-OBESITY AGENT IN THE FEED OF ADULTS AFRICAN MUD CATFISH (*Clarias gariepinus*)

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African sharp toothed catfish *Clarias gariepinus* is the most widely cultivated species in Nigeria due to its ruggedness and the ease with which its breeding is manipulated. It is notable for its high fat content hence it is classified as a fatty fish. Obesity was induced in the fish by High Fat Diet (HFD) for four weeks and subsequently treated with diets containing the calyx extract of *Hibiscus sabdariffa* at three different concentrations (400mg/kg A1, 800mg/kg A2, and 1200mg/kg A3) with other control groups being Standard Drug Diet (SDD), Copens Control Diet (CCD) and HFD for five weeks. This study seeks a panacea to the problem of high fat content in Catfish by incorporating the extract of the calyces of *Hibiscus sabdariffa* which had been proven to be effective in combating obesity in humans and rodents.

For the first four weeks, the HFD fish had the highest MWG and %MWG of  $21.3 \pm 10.69\text{g}$  and  $10.03\%$  respectively, while the control group was  $5.99 \pm 16.43$  and  $2.99\%$  respectively. Fish fed the HFD also had the best SGR of  $132.75 \pm 66.37\text{g}$  compared to the control with SGR of  $37 \pm 102.09$ . At the end of the five weeks of treatment, fish fed the A3 diet had had the highest weight loss with MWG and %MWG of  $18.06 \pm 12.66\text{g}$  and  $0.53 \pm 0.76\%$  followed by standard drug control with MWG and %MWG of  $19.60 \pm 0.02$  &  $1.42 \pm 0.01$  respectively. The study suggests that high fat diet can induce obesity in fish and excessive fat can be reduced by the calyx extract of *Hibiscus sabdariffa* at concentration of 1200mg per 1kg of feed. The study revealed the preference of captured to farm raised catfish among adults and the ageing group of the society. The rejection of aquaculture fish by this segment of the population is to a larger percentage attributed to commercial feed with which the fish are fed, consequently leading to high fat level in the fish muscle.

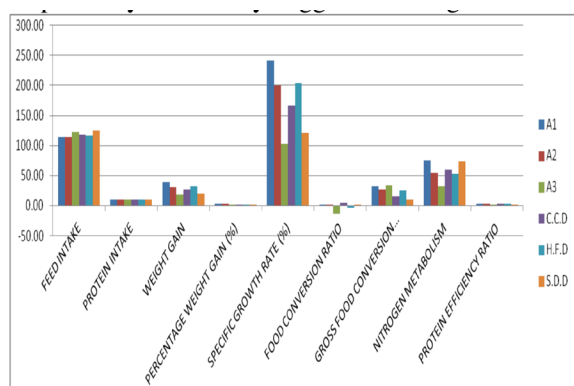


Figure 1: Mean growth performance of *Clarias gariepinus* fed six different experimental diets over a period of five weeks

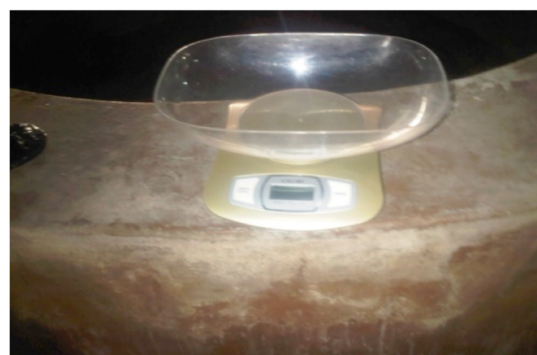


Figure 2: Camry electronic scale (model EK3650/EK3651)

Table 1: Mean values of growth parameters of Copens control (CCD) diet and high fat diet (HFD)

| Parameters | A1                  | A2                   | A3                   | CCD                  | HFD                  | SDD                  |
|------------|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| FI(g)      | $113.64 \pm 9.39^a$ | $113.51 \pm 3.91^a$  | $122.27 \pm 10.64^a$ | $118.73 \pm 0.04^a$  | $117.45 \pm 0.04^a$  | $124.87 \pm 0.23^a$  |
| PI         | $10.26 \pm 1.01^a$  | $9.88 \pm 0.27^a$    | $10.60 \pm 0.34^a$   | $9.96 \pm 0.03^a$    | $9.87 \pm 0.00^a$    | $10.46 \pm 0.10^a$   |
| WTG        | $39.56 \pm 31.45^a$ | $30.69 \pm 21.25^a$  | $18.06 \pm 12.66^a$  | $26.63 \pm 0.21^a$   | $32.93 \pm 0.61^a$   | $19.60 \pm 0.20^a$   |
| %WTG       | $3.22 \pm 1.46^a$   | $2.71 \pm 1.43^b$    | $0.53 \pm 0.76^{ab}$ | $2.02 \pm 0.03^{ac}$ | $2.54 \pm 0.01^{ab}$ | $1.42 \pm 0.00^{ac}$ |
| SGR        | $242 \pm 96.23^a$   | $200.52 \pm 58.95^a$ | $102.95 \pm 80.72^a$ | $166.33 \pm 0.14^a$  | $203.57 \pm 0.11^a$  | $121.60 \pm 0.0^a$   |
| FCR        | $0.82 \pm 5.68^a$   | $0.90 \pm 1.04^a$    | $-12.93 \pm 4.28$    | $4.29 \pm 0.03^a$    | $-3.72 \pm 0.58^a$   | $0.58 \pm 0.20^a$    |
| GFCR       | $32.60 \pm 21.31^a$ | $27.09 \pm 23.98^a$  | $33.84 \pm 38.45^a$  | $16.20 \pm 0.02^a$   | $25.19 \pm 0.01^a$   | $10.67 \pm 0.12^a$   |
| Nm         | $74.77 \pm 59.45^a$ | $54.41 \pm 38.87^a$  | $32.81 \pm 25.9^a$   | $59.33 \pm 0.06^a$   | $53.29 \pm 0.04^a$   | $74.10 \pm 0.03^a$   |
| PER        | $3.62 \pm 2.71^a$   | $3.29 \pm 2.64^a$    | $2.45 \pm 1.53^a$    | $3.11 \pm 0.03^a$    | $3.06 \pm 0.02^a$    | $1.41 \pm 0.02^a$    |

## MARINE FISHERIES POLICY IN CHINA: PAST, PRESENT AND FUTURE

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China is one of the world's largest and most important marine and fishery countries. In past consecutive 26 years, the output of Chinese aquatic products ranked first in the world. In 2016, the total output of Chinese aquatic products is 69 million tons, which accounting for about 40% of the world's output of aquatic products. China is also the largest marine fishing and aquaculture country in the world, respectively accounting for about 18% of the total world marine catch and 61% of the total world aquaculture. The development and Policies of Chinese fisheries play an important role in the global food supply(Fig.1) . Since China launched the strategy of “farming as the main and catch as the auxiliary” in 1980s, many researchers pay more attention to Chinese aquaculture, but not to know much about marine catch in China

The goal of this paper is to give an overview of changes of marine fisheries policy in China with the development of Chinese fishery. According to development characteristics of fishery in past several decades, marine policies underwent five historical states which underwent five historical stages which were sprouting(1950-1970), generating(1971-1980),developing(1981-1990), climaxing(1991-2000), and stabilizing (2001-2015). A series of policies and measures have been worked out by Fishery Administration Bureau and The State Oceanic Administration, such as, fishing licensing system, Regulation of summer fishing moratorium (the period and marine sea area of fishing ban), Double control the total engine power and the number of fishing vessels, Minimum mesh size regulation, Stock enhancement, Management system of national marine fish nature reserve, Subsidy policy of fuel, Mutual insurance system, Measures of transferring fishermen's job, etc. Although many achievements have been made in fishery management, the offshore fishery resources are declining and overfishing. In the current situation, the problem of how to balance the output of marine catch and sustainable development of marine fishery resources is becoming increasingly urgent. So, the future direction of policy adjustment in China will still aim at decreasing the total marine catch under the condition of increasing the fishermen's income more than before.

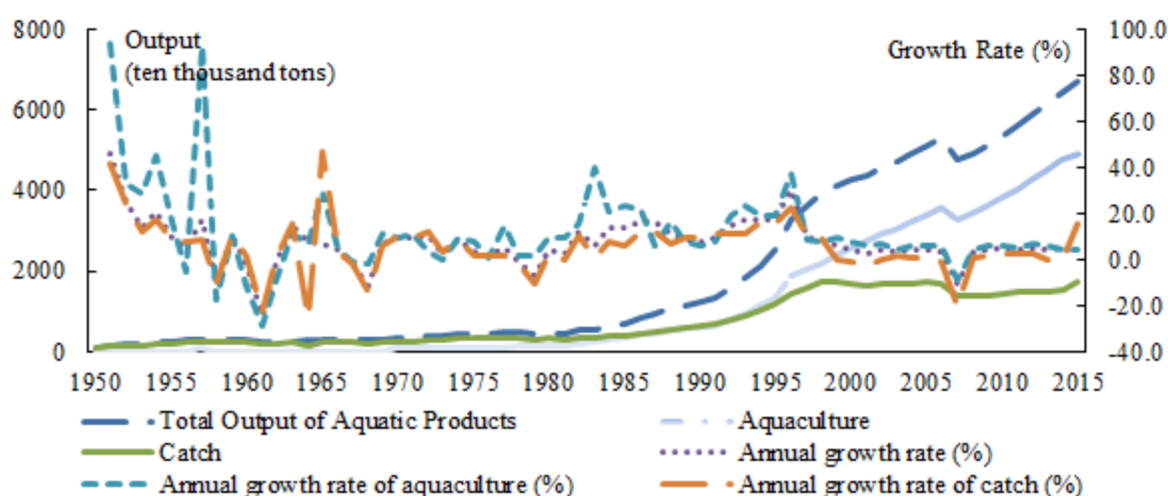


Fig.1. The Output of Aquatic Products in China in 1950-2015.

Data from: China fishery statistics yearbook

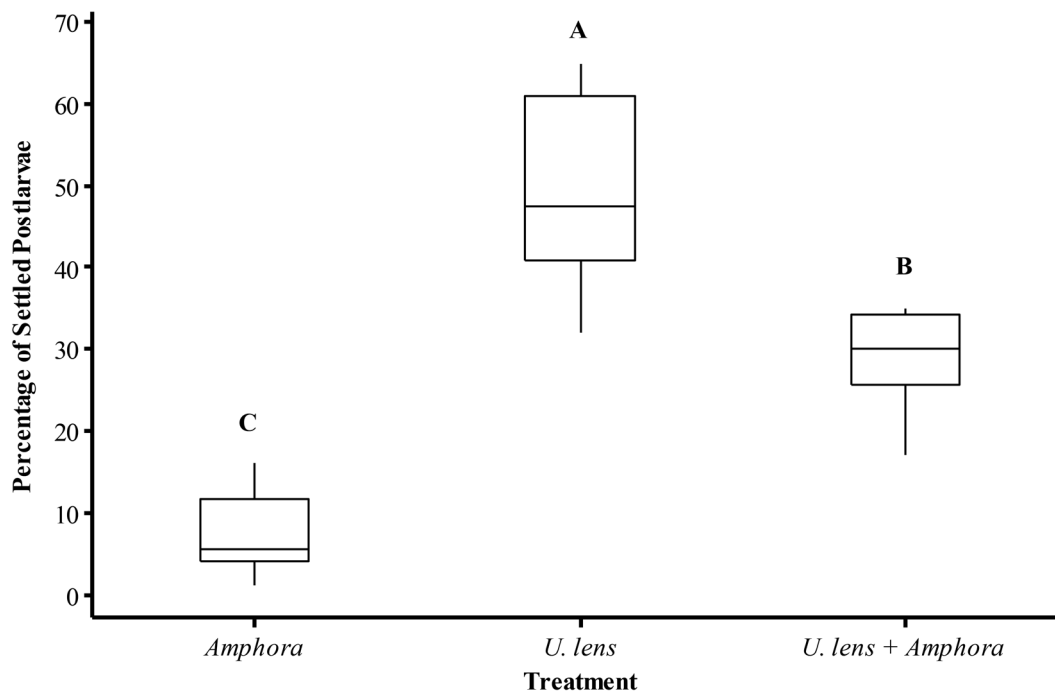


## THE APPLICATION OF *Ulvella lens* TO THE EUROPEAN SEA URCHIN INDUSTRY

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The Japanese development of mass culture techniques for *Ulvella lens* during the 1980s for aquaculture has stimulated the development of rearing techniques for abalone and sea urchins in producing countries. However, since the late 1980s, there has not been any in-depth evaluation of culture methods for *U. lens*, nor the development of a new robust method for aquaculture operators. The use of this known inducer for settlement of veliger larvae has not been employed on a commercial scale in Ireland or in European aquaculture systems. The new methodology described has produced a threefold increase in the settlement of sea urchins (*Paracentrotus lividus*) when compared to benthic diatoms and is recommended for adoption by the European aquaculture industry. This is the first evaluation of settlement inducers that includes *U. lens* for the urchin species *P. lividus*. The findings are readily transferrable to echinoderm aquaculture as echinoderms share similar life histories with other commercially important invertebrates. Industry application of these results within commercial *P. lividus* hatcheries, can increase productivity and reduce costs, by increasing the production capacity of commercial aquaculture enterprises without having to change existing nursery facilities or equipment. Although these results demonstrate advancement of commercial sea urchin aquaculture, achieved through transfer of technologies established in invertebrate aquaculture, the long-term use of *U. lens* requires further research to establish post-settlement growth and survival rates of *P. lividus* at commercial scales and under commercial conditions. *U. lens* is a highly successful inducer of *P. lividus* larval settlement and is an important advancement in the culture of this commercial sea urchin species.





## SEMI-INTENSIVE POLYCULTURE OF CLIMBING PERCH WITH INDIAN CARPS

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Air-breathing fishes provide a significant advantage for pond culture, as they tend to be resilient to harsh conditions, particularly during periods of low-oxygen, which can occur with high temperatures, drought or poor water quality. Currently, in Bangladesh production of Koi (*Anabas testudineus*, climbing perch) is limited to monoculture systems with intensive stocking and use of commercial-grade feeds. As feed can comprise up to 60% of total production costs, the current culture practices for these fish limit participation by small homesteads and therefore comprise a significant impediment to further expansion of this industry. Further, the use of high-levels of feed inputs has led to a persistent deterioration of pond water quality. We assessed 1) the feasibility and profitability of semi-intensive polyculture of Koi with Indian carps (Rohu and Catla) in ponds, and 2) whether Koi-carp polyculture is best with Rohu or Catla alone or when the two are combined. The study was carried out for 120 days in ponds at Bangladesh Agricultural University. The experiment consisted of three treatments (T1, T2, T3) with four replicates each. Koi were stocked at the same density in all groups (5/m<sup>2</sup>) and feed was applied based on Koi biomass at 50% (10% - 2.5% bw/day) of the rate typically used by industry. Treatment 1 was stocked with 0.8 Rohu/m<sup>2</sup> and 0.2 Catla/m<sup>2</sup>, T2 with 1.0 Rohu/m<sup>2</sup>, and T3 with 1.0 Catla/m<sup>2</sup>. All ponds were fertilized weekly to enhance productivity of natural food for carps.

Weekly and fortnightly water quality parameters were measured and did not vary significantly the three treatment groups. There was no difference in weight gain or specific growth rate for any species between treatments or for survival rate for Koi. The survival rate for Rohu in T1 was significantly higher than T2 and for Catla in T1 than in T3. Both gross and net production parameters were significantly higher for Koi in T3 than in T1 or T2. Catla grown at a stocking rate of 1.0 fish/m<sup>2</sup> (T3) resulted in production parameters that were significantly higher than when stocked at 0.2 fish/m<sup>2</sup> (T1). There was no significant difference in production of Rohu between treatments. Feed conversion ratio (FCR) and BCR were similar among treatments. In conclusion, the results indicate that Koi can be polycultured with carps and this system produces significant positive returns on investment when fish are fed at half ration in fertilized ponds. While the best polyculture production may occur when Koi are solely cultured with Catla, Koi can also be cultured with either Rohu alone or both Rohu and Catla at a 4:1 ratio. Future studies are required to directly compare Koi monoculture and Koi-carp polyculture and the impacts of feed and fertilization inputs.

Table 1. Production parameters. Values are mean ± SD. Values with different letters are significantly different ( $P < 0.05$ ). NA = not applicable.

|                                    | Treatment 1                  | Treatment 2              | Treatment 3              |
|------------------------------------|------------------------------|--------------------------|--------------------------|
| <b>Koi (<i>A. testudineus</i>)</b> |                              |                          |                          |
| Stocking Weight (g)                | 2.94±0.87                    | 2.94±0.87                | 2.94±0.87                |
| Harvest Weight (g)                 | 129.24±36.27                 | 148.39±28.39             | 144.13±15.79             |
| Survival Rate (%)                  | 72.64±23.46                  | 64.36±9.92               | 77.36±11.74              |
| Specific Growth Rate (%/day)       | 3.13±0.25                    | 3.26±0.17                | 3.24± 0.09               |
| Gross Production (kg/ha)           | 4,325 ± 390 <sup>b</sup>     | 4,618 ± 374 <sup>b</sup> | 5,459 ± 532 <sup>a</sup> |
| Net Production (kg/ha)             | 4,219.25±378.42 <sup>b</sup> | 4,524 ± 381 <sup>b</sup> | 5,346 ± 521 <sup>a</sup> |
| <b>Rohu (<i>L. rohita</i>)</b>     |                              |                          |                          |
| Net Production (kg/ha)             | 1,102 ± 267                  | 1,066 ± 186              | NA                       |
| <b>Catla (<i>G. catla</i>)</b>     |                              |                          |                          |
| Net Production (kg/ha)             | 344 ± 120 <sup>b</sup>       | NA                       | 922 ± 333 <sup>a</sup>   |

## MINERAL CONTENTS EVALUATION OF SOME SHELLFISHES FROM BUGUMA CREEK, RIVERS STATE, NIGERIA

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Shellfishes have become a worldwide delicacy among seafood lovers. They are high in essential nutrients and are extremely beneficial to health (Soundarapandian *et al.*, 2013). Invertebrate animals such as periwinkles, rock snails, oysters and crabs which possess exoskeleton called shells which may be single or double over the body are called shellfishes. They are mainly algae and diatom feeders (Okon, 1987). Periwinkle like other shellfishes has a high commercial value in the Niger Delta area of Nigeria. Their value can be compared with those of domestic livestock and fish.

They are widely eaten because they are nutritious with good mineral content (Ogogo, 2004), by the people of the Niger Delta region of Nigeria. The study was conducted to evaluate the mineral composition of Periwinkles (*Tympanotonus fuscatus*), Rock snails (*Thais coronata*), Mangrove oysters (*Crassostrea gasar*) and Land crabs (*Cardisoma guanhumi*) found in the marine environment of Buguma Creek in Rivers State, Nigeria.

Copper, iron, manganese, calcium and sodium were quantified using Atomic Absorption Spectrophotometric method (AOAC, 2005). The results were expressed in mg/100gm of specimen respectively. All the determinations were in triplicates and the same was repeated for all the species and the values recorded. Statistically data was analyzed using a one-way ANOVA and Duncan's multiple range tests to compare mean values of the samples and to avoid error. Mineral contents of the edible parts of the shellfishes are presented in Table 1. The concentration of the minerals varied between the different species from the Creek. The result confirmed that the shellfishes are rich sources of minerals.

**Table 1:** Mean value of the mineral composition (mg/100g) of the flesh of the shell fishes

| Minerals         | <i>T. fuscatus</i>      | <i>T. coronata</i>      | <i>C. gasar</i>        | <i>C. guanhumi</i>       | <i>C. amnicola</i>       |
|------------------|-------------------------|-------------------------|------------------------|--------------------------|--------------------------|
| <b>Copper</b>    | 3.38±0.05 <sup>b</sup>  | 9.66± 0.02 <sup>a</sup> | 0.56±0.01 <sup>d</sup> | 1.97 ± 0.01 <sup>c</sup> | 0.54 ± 0.02 <sup>d</sup> |
| <b>Iron</b>      | 6.78±0.06 <sup>c</sup>  | 14.6± 0.05 <sup>c</sup> | 37.8±0.01 <sup>a</sup> | 24.6 ±0.31 <sup>b</sup>  | 9.72± 0.03 <sup>d</sup>  |
| <b>Manganese</b> | 15.9± 0.03 <sup>c</sup> | 19.2± 0.01 <sup>d</sup> | 98.4±0.02 <sup>c</sup> | 144.8±0.05 <sup>a</sup>  | 142.7±0.63 <sup>b</sup>  |
| <b>Calcium</b>   | 55.8± 0.19 <sup>c</sup> | 139.2±0.58 <sup>c</sup> | 69.6±0.04 <sup>d</sup> | 225.1±17.8 <sup>a</sup>  | 211.3±0.68 <sup>b</sup>  |
| <b>Sodium</b>    | 0.22± 0.01 <sup>c</sup> | 2.11± 0.01 <sup>a</sup> | 0.74±0.03 <sup>c</sup> | 0.47 ±0.01 <sup>d</sup>  | 1.03 ± 0.02 <sup>b</sup> |

\* In each row, means with a common letter are not significantly different (P>0.05)

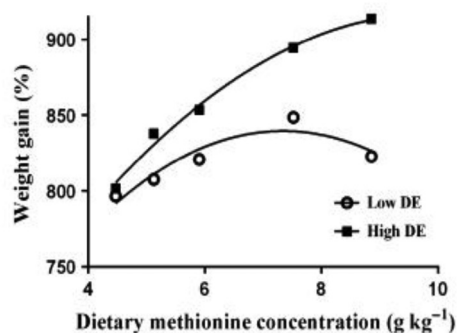
## THE EFFECT OF DIETARY METHIONINE CONCENTRATIONS ON GROWTH PERFORMANCE OF JUVENILE NILE TILAPIA (*Oreochromis niloticus*) FED DIETS WITH TWO DIFFERENT DIGESTIBLE ENERGY LEVELS

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A growth trial was conducted to examine the effect of dietary digestible energy (DE) content on methionine (Met) utilization and requirement in juvenile Nile tilapia (*Oreochromis niloticus*). Ten iso-nitrogenous (288 g kg<sup>-1</sup> protein) practical diets, with two DE levels (10.9 MJ kg<sup>-1</sup>; 12.4 MJ kg<sup>-1</sup>) and five methionine supplementation levels (0, 1, 2, 4 and 6 g kg<sup>-1</sup>), were hand-fed twice daily to triplicate groups of Nile tilapia (initial body weight 8.95 ± 0.06 g) for 8 weeks.

Weight gain (WG) and specific growth rate (SGR) increased significantly with increasing dietary methionine concentration at the same DE content ( $P < 0.001$ ). At the same dietary methionine level, WG and SGR of fish fed high-DE diets were significantly higher than that of fish fed low-DE diets ( $P = 0.0001$ ), although no interaction was found between dietary DE and methionine supplementation. Based on quadratic regression analysis between dietary methionine concentration and weight gain, optimal methionine requirement for maximum growth, expressed as g Met required kg<sup>-1</sup> diet (low- versus high-DE diets), increased as diet DE concentration increased (7.34 versus 9.90 g kg<sup>-1</sup> diet, respectively; with cysteine 4.70 g kg<sup>-1</sup> diet). The results indicated that diet DE content affects methionine utilization and requirement in juvenile Nile tilapia, fish fed high-DE diets required more methionine for maximum growth.



**Figure 1** Weight gain (WG) responses to dietary methionine concentrations (g kg<sup>-1</sup>) at low (10.9 MJ kg<sup>-1</sup> DE) and high (12.4 MJ kg<sup>-1</sup> DE) energy levels using quadratic regression analysis for 8 weeks. The equation that best described the response is follows:  $Y = 527 + 85.2X - 5.81X^2$  ( $R^2 = 0.88$ ,  $P < 0.0001$ ) at low DE level;  $Y = 546 + 75.1X - 3.79X^2$  ( $R^2 = 0.99$ ,  $P < 0.0001$ ) at high DE level. Estimates of methionine requirements (g kg<sup>-1</sup> diet, low versus high diet DE diets) were: 7.34 versus 9.90, respectively.

## CRYOPRESERVATION PROCEDURES FOR LOCAL ALGAL MASTERS STOCK USED IN AQUACULTURE

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The storage and maintenance of large numbers of single strain algal collections can often be a tedious, costly and a timeless affair, unless the cultures are regularly being used or up scaled for production. It would therefore be beneficial to have a reliable method of long term storage as a means of ensuring the integrity of algal master stocks. Cryopreservation has been shown to be particularly useful in the long term storage of algal material/cultures.

In this study we investigate the effects of cooling and low temperature damage on microalgal strains regularly used by the South African aquaculture industry. Variables tested included cooling rates, the use of various types and concentrations of cryoprotectants, final temperatures attained, thawing temperatures, storage times as well as algal species (*Chaetoceros muelleri*, *Nannochloropsis oculata*, *Pavlova lutheri*, *Isochrysis galbana*, *Amphora* sp., *Cocconeis* sp., *Navicula Jeffreyae* and *Nitzschia constricta*). Successful cryopreservation was measured as the ability to successfully re-suspend a preserved algal culture, cell counts were used to measure algal growth. This information will be particularly useful in determining the most appropriate means of preserving specific algal species used in aquaculture for long term storage and assist with the maintenance of key species utilized by local producers.

## ROLE OF SOUND PRODUCTION IN THE REARED SCIAENID *Sciaenops ocellatus* DURING THE BREEDING SEASON IN AQUACULTURE DEVICE

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The red drum *Sciaenops ocellatus* is an estuarine-dependent sciaenid originating from the south of the United States. Important commercial fisheries have resulted in a decline of wild populations. Since the 1980s, this species has been introduced in Martinique and in different parts of the world as a new species for marine aquaculture. Males *Sciaenops ocellatus* are known for their ability to make low frequency calls during reproduction period. Sound production results from the fast contraction of sonic muscles that dorsally surround the swimbladder. The hypothesis supporting this study is that sounds could be used to enhance farming and aquaculture efficiency. This study aimed to understand the biology of the sound production in *Sciaenops ocellatus* and its exact role during the reproductive period.

A total 6-months study period, extending over two spawning seasons, was conducted on farmed fish. Sexually mature adults *S. ocellatus* were recorded in fiberglass tanks for 1 min 30 every 30 minutes. A group of 12 males and 8 females were held in a 12.5 m<sup>3</sup> tank for 63 days. Seven couples were each held in 6 m<sup>3</sup> tanks for a period of 22 to 71 days. In the group, the photoperiod was maintained to resemble a natural reproductive period (9 hours light). Couples were exposed to the natural daylight (11 hours light).

The major acoustic activity took place during the night. Sounds are composed of a variable number of pulses which tended to be greater during the night. In the group, the mean number of pulses per call was significantly higher during spawning nights ( $p < 0.0001$ ) and the peak of acoustic activity corresponds to egg laying events. In experiments using couples, it was observed that fertilization of the spawn only occurred on nights with male sounds. Moreover, it seems that the number of sounds produced plays a role in the number of eggs fertilized. These results indicate that sounds could be used not only as a male-female attraction, but also as a synchronizing signal for gamete emission and fertilization.



FIGURE 1. Acoustic and spawn features of two spontaneous reproductions, separated from 30 days, observed in a couple.

## **NUTRITIOUS POND PROJECT: NEW STEPS IN DEVELOPING A SUSTAINABLE AQUACULTURE PRODUCTION SYSTEM THROUGH DIET FORMULATION**

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With ongoing growth of the world population, we are facing an increasing demand for seafood. While global fisheries reaches its maximum potential, there is pressure on aquaculture to increase production which brings along serious issues concerning sustainability. Over the last decades, intensification of aquaculture has resulted in increased water use, disease incidence, antibiotic and chemical use, reduced fish health and quality, reduced production predictability, and massive output of polluting waste materials into the environment. Nowadays the sector has become largely dependent on external nutrient resources instead of relying on the natural carrying capacity of ponds. Limiting resources such as fish meal as source of specific amino acids, or fish oil as source of omega-3 fatty acids, inhibit expansion possibilities of the aquaculture sector in the near future. Without compromising on current production rates, the nutritious pond project aims on reducing dependency of aquaculture on external nutrient resources by stimulating natural food production in the pond through improving nutrient balances of formulated feeds. Optimizing the fertilizing properties of formulated feeds may additionally result in higher turnover rates and reduced waste output. By gaining more knowledge on natural food production and nutrient transfer in the pond, an innovative yet easier production system and cheaper feed will be developed and implemented. This research focusses on generating insight in 1) the dynamic processes related to production and transfer of omega-3 fatty acids by algae in the pond, and 2) ways to balance the ratio between algae and bacteria for optimal decomposition, maintenance of water quality and nutritive value of fish and shrimp. A series of experiments has been performed whereby feed formulations differed in ratios and content of carbon, phosphorus and nitrogen, and content of fishmeal and fish oil. These experiments took place in both controlled indoor artificial mesocosm systems, as well as in outdoor tilapia and shrimp ponds. Preliminary outcomes show that full exclusion of fishmeal and fish oil from formulated feed did not affect growth performance and survival, indicating sufficient contribution of omega-3 fatty acids from natural production in the pond. Also, by balancing nutrient ratios of formulated diets, the contribution of natural food to fish production is enhanced. This approach allowed to lower feed input in ponds by 40% without a negative effect on growth and survival of the culture species. Currently lab analyses are being performed and results will be ready to be presented at World Aquaculture 2017 in Cape Town. Stable isotope outcomes will highlight the effect of dietary treatments on natural food selection and contribution to growth of the culture species. Stoichiometry and fatty acids profiles will describe the fertilizing properties of the dietary treatment diets.

## CAPTIVE BREEDING AND LARVAL REARING SUCCESS OF SPINY LOBSTER *Jasus lalandii*

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The rock lobster *Jasus Lalandii* forms the basis of important fisheries in South Africa and Namibia. Their long pelagic larval phyllosoma phase (12-24 months) has been studied extensively and there remain many unresolved issues surrounding the retention and recruitment into local populations. Palinurid (spiny) lobsters are found throughout the world's oceans between latitudes 45 deg N and 45 deg S. They form the basis of many important commercial fisheries. The West Coast Rock Lobster *Jasus Lalandii* is a species of spiny lobster found off the coast of Southern Africa. It primarily occurs in shallow waters from Cape Cross in Namibia to Algoa Bay in South Africa, where it can usually be found on the rocky sea floor. West Coast Rock Lobsters (WCRL) are slow growing, long-living animals listed on NEMBA (the National Environmental Management Biodiversity Act of 2004) as a threatened and protected species. This is a species of high conservation value and national importance that requires national protection. From 1970 onwards, there were declines in the population growth rate of the species. In 1990/1991 season TAC and 3790 tonnes, in 1992/1993 it was 2400 tonnes and in 1995/1996 it was 1500 tonnes. Since these reductions were implemented there appears to be a steady stock recovery which resulted in a steady increase in the TAC. In 2011/2012 the TAC was 2425 tonnes. It is postulated that at the current rate the WCRL should continue to build, however, the 2012/2013 TAC was not set in accordance with the scientific target recommendations raising new fears on the future of wild stocks. The research was done with the objective to reproduce the species in captivity

Six pairs of wild caught, captive brood-stock (2012) of spiny lobster *Jasus Lalandii*, mated successfully and produced more than 28 spawning's over a three year period (2014-2016). Acclimatised female brood-stocks produced spawning's once a year during Spring (between September and November). Ovigerous females were transferred to a Larval release tank designed to mimic release conditions approximately one month ahead of estimated release dates. Conditions were manipulated to induce release response in the release tanks each morning ahead of sunrise around the release dates. Naupliosoma were released in two or three batches on successive days. The number of naupliosoma hatched and released ranged from a few hundred to a few hundred thousand with female breeder weights ranging from 350g to 550g. The eggs hatched at sunrise after 80-90 days into naupliosoma larvae which swam to the surface using their antennae/setae. They slowly 'unfolded' into phyllosoma on exposure to sufficient UV/light/surface warmth. The phyllosoma's phototactic response entraining them into the direction of sunlight and current. Phyllosoma appear as tiny, highly transparent, spider-like larvae with long, spiny/hairy, dotted- pink pereioopods. The phyllosoma were harvested in a larval 'vortex' collection chute whereafter they were transferred to the larval rearing vessel.



## MACHINE LEARNING CLASSIFICATION OF RUSSIAN STURGEON GENDER FROM PLASMA METABOLOMICS DATA

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Russian sturgeons are long-lived fish native to the Black Sea and Caspian Sea as well as their river basins. These ancient fish are being farmed to meet demands for their meat as well as for their prized caviar. Presently, there is no practical and accurate method to identify the gender of these sturgeon well in advance of maturity, which can take as long as 8 to 16 years. Farmers would benefit greatly from identifying gender in young fish in order to separate females from males for caviar production. A multi-platform, global small molecule profiling (metabolomics) analysis was conducted on sturgeon blood plasma sampled from immature fish of known gender at 10 (N=7 fish each gender) and 22 (N=25 fish each gender) months of age. A total of 483 named biochemicals were quantified in the plasma and machine learning support vector machines (SVMs) were used to model differences in the patterns of biochemicals between males and females in each of the age groups. Sensitivity analysis was performed to rank biochemicals in order of importance in the machine learning classification procedure and dimensionality of the data was reduced using sequential SVM models to identify two lists of 15 compounds that are highly predictive of gender either at 10 months or 22 months of age, respectively. Three of the 15 compounds important to differentiating gender of 10 month-old fish and four of the 15 compounds important to differentiating gender of 22 month-old fish significantly differed between males and females (ANOVA,  $p \leq 0.05$ ). The majority of compounds important for gender identification in Russian sturgeon represented active growth metabolic pathways and transport and cellular uptake of nutrients. Although these pathways were similarly important for differentiating male and female fish at 10 or 22 months of age, differences in the particular biochemicals observed between genders in 10 month-old fish were not important for gender classification at 22 months of age and vice versa. Therefore, the markers are not universally applicable to fish of differing age groups. Additionally, no single biochemical appeared to provide for adequate gender identification in light of the observed biological variation. The findings show that machine learning pattern recognition can be used to accurately classify the gender of immature sturgeons using plasma metabolite data, though potential gender biomarkers will need to be further validated in larger groups of individuals.

## HISTOLOGICAL EXAMINATION OF REPRODUCTIVE MATURITY OF SEX-REVERSED RAINBOW TROUT *Oncorhynchus mykiss* FEMALES DURING SPAWNING SEASON

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Assessment of the fish maturity is one of the most important issues in artificial reproduction, especially for fish obtained due to genomic manipulation combined with hormonal therapy such a sex-reversed rainbow trout. Matured sex-reversed females (neomales) are morphologically indistinguishable from normal males, but usually have less well developed testes, and lack of spermatid ducts. In the present study, original new histological scale has been used to assess stage of gonad maturity in the rainbow trout neomales during spawning season.

Histological analyzes were made on 132 neomales with an average body weight of  $1493 \pm 194$  g and the average body length of  $44.9 \pm 6.1$  cm. The study was carried out in five periods of spawning season, based on percent of ready for ovulation females in the broodstock: just before (January 13<sup>th</sup>); at the beginning (10% ovulated fish – February 28<sup>th</sup>); peak of spawning (about 50% ovulated fish – March 22<sup>nd</sup>); at the end (about 90% ovulated fish – April 16<sup>th</sup>); immediately after the end (May 11<sup>th</sup>). Histological protocol with embedding, cutting and staining of testes pieces with HE method were used. Analyses of gonads cross-sections were made with an LEICA DM 3000 light microscope and micro image computer analysis software LEICA QWin Pro (LEICA Microsystems AG, Heerbrugg, Switzerland).

The gonad maturity of rainbow trout neomales during analyzed season was characterized by high variation. Therefore, histological scale consisted five stages of testes maturity was proposed: (I) – characterized by regular size of seminiferous tubules fulfilled with spermatids and spermatozoa (Fig. 1A). (II) – tubules in different size and their walls isolated by space between the germ cells located inside structures (Fig. 1B). (III) – unsettled the spatial structure of seminiferous tubules. Part of the walls were perforated and spermatozoa filled most of the available testes space (Fig. 1C). (IV) – spermiation phase – most of tubules lose their regular shape and are completely or partially free of spermatozoa. In cross-sections visible single spermatogonia – characterized for first stages of spermatogenesis (Fig. 1D). (V) – seminiferous tubules mostly empty with irregular spatial structure. The tubules walls had increased thickness where appeared a large number of spermatogonia and primary spermatocytes (Fig. 1E).

Results could help to improve the production technology of all-female populations of rainbow trout in aquaculture.

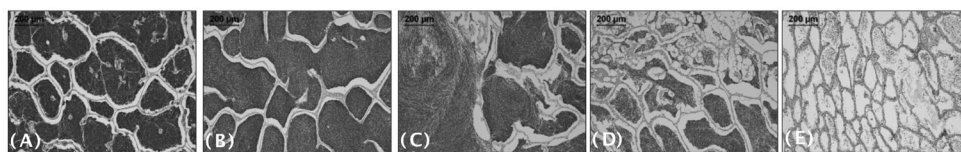


Fig. 1. Cross-sections of rainbow trout neomales gonads during spawning season.

## HOW STAKEHOLDER PERCEPTIONS CAN INSPIRE CLIMATE CHANGE ADAPTATION STRATEGIES FOR SEA FOOD INDUSTRIES

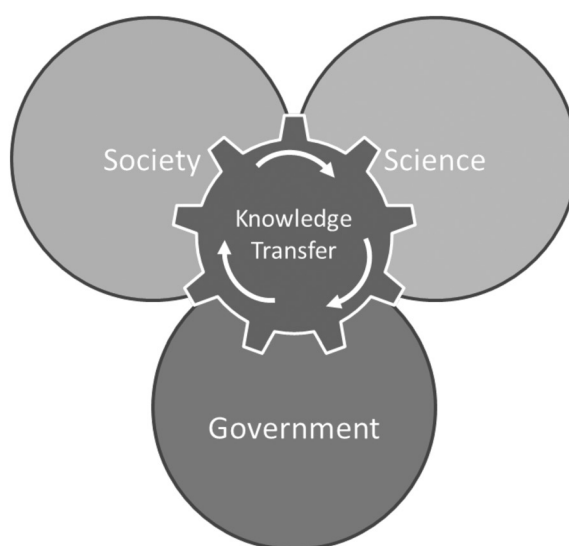
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Worldwide, in many coastal areas the local populations and economies depend on the goods and services the marine ecosystems provide. The pressure on those ecosystems has continuously increased in the last decades due to climate change and intensified anthropogenic use. Especially in coastal regions environmental changes, like increased storm intensity and frequency, changes in precipitation, increasing sea surface temperatures and ocean acidification, have tremendous effects on sea food production. Despite the global nature of this problem, we will have to tackle its causes and adapt to the coming changes on local scales, requiring local adaptation strategies and involvement of all relevant stakeholders. This study takes a trans-disciplinary approach to identify the threats as well as the opportunities climate change poses for the sea food industries on the German North Sea Coast.

Two workshops were conducted to approach and involve key stakeholders and to tap their knowledge bases to obtain a new problem focus. The first workshop targeted a broad audience of local stakeholders from fisheries, fish processing, local government and tourism associations to NGOs, while the second workshops target audience were early stage marine researchers. Even though the participants' perceptions were different in the two workshops and many different topics of importance were identified, they both noticed a great demand for improved communication between different stakeholder groups to enable the development of a local adaptation strategy for the sea food industries.

One of the most important lessons learned from the workshops and stakeholder involvement is that a direct and clear transfer of knowledge between the different parties is crucial to increase the understanding of the process and impacts of climate change (Fig. 1). Furthermore novel inter- and trans-disciplinary approaches are needed in climate change research and management for the sea food industries including aquaculture and fisheries.



**Figure 1 The role of knowledge transfer in the development of climate change adaptation strategies**

## **IMPACT OF MARICULTURE INFORMATION DISSEMINATION TO THE LOCAL COASTAL COMMUNITIES IN KENYA**

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The study was conducted to investigate the awareness level of aquaculture and impact of trainings to the local communities from Mtwapa, Kilifi, Malindi and Lamu in coastal Kenya.

A questionnaire was used to collect information and a sample size of 30 communities randomly selected from the four local villages.

Data analysis was carried out using Ms excel where the respondent's views in the pre and post training period were tabulated and displayed in form of percentages.

Using correspondence analysis technique, participants views were characterized on knowledge capacity of questions administered in a single plot (Bi-plot) vis a vis the selected ranks in form of a likert scale where farmers indicates YES, LITTLE or NO as per their understanding of the question.

50% of the new farmers had no idea about fish farming, 43% had little idea and 7% never heard about fish farming. On practicing farmers, 95% knew what fish farming was all about while 5% had little idea about it.

55% of practicing fish farmers involved themselves in fish farming.

It's recommended that extension agents should reach out to communities and pass useful information to them in order to increase farming profitability.

## INTERTIDAL MUDFLAT FISH CULTURE IN CHINA

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The two intertidal mudflat fishes, the mudskipper *Boleophthalmus pectinirostris* (Fig.1) and Chinese black sleeper *Bostrichthys sinenses* (Fig.2), have been farmed in southeastern China since the 1980's. They are commercially important marine fish species with high market prices. At present the farmed area of the former reaches 7,000 ha, producing 6 million kg, valuing  $\square$ 720 million; and the farmed area of the latter reaches 2,000 ha, producing 7.5 million kg, valuing  $\square$ 750 million annually. Farming these two fish species is a profitable industry.

There are two farming systems for the mudskipper, monoculture in earth pond and polyculture in intertidal mudflat net-enclosure, while the Chinese black sleeper mariculture is carried out in earth pond either in monoculture mode or in polyculture mode with shrimps. The stocking densities for the mudskipper and Chinese black sleeper in monoculture mode are 45,000-75,000 ind./ha. The resource of the wild mudskipper fry is available and that of the wild Chinese black sleeper fry is scarce at present. Therefore, most of the mudskipper fry for farming are captured from intertidal mudflat by professional collectors, although the larval rearing of this species has been succeeded, and most of the Chinese black sleeper fry for farming are produced in hatcheries in the spawning seasons.

The mudskipper is an herbivorous species, feeding mainly on benthic diatoms, copepods and protozoa, while the Chinese black sleeper is a carnivorous one, feeding on shrimps, crabs and other small fish species. In the farming practice, no artificial diets are provided for the mudskipper; the most important step of its farming management is to grow the benthic algae regularly for the fish by the application of fertilizers. Hence, the mudskipper farming is a less investment and pollution-free industry. On the other hands, the Chinese black sleeper is fed mainly with trash fishes and a small amount of artificial diets. The total investment of the Chinese black sleeper farming is much higher than that of the mudskipper. Both species take 12-18 months to grow to the marketable size with survival rates of 65-85%, based on stocking sizes of fry. Lower water salinities at 10-15 are suitable for these two fish species to grow.



Fig. 1 *Boleophthalmus pectinirostris*



Fig.2 *Bostrichthys sinenses*

**THE INFLUENCE OF AGONISTIC BEHAVIOR ON REPRODUCTIVE PERFORMANCE IN CAPTIVITY REARED FEMALES IN AN ENDANGERED SILURIFORM SPECIES, *Steindachneridion parahybae***

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The Neotropical teleost *Steindachneridion parahybae* is a gonochoristic medium-sized siluriform species, an important migratory (reophilic fish), ichthyophagous and freshwater fish, endemic to the Paraíba do Sul River Basin (Brazil), seriously endangered in this basin and regionally extinct in São Paulo State. The scarce biological information of *S. parahybae* and the critically endangered situation highlight the importance of increasing the knowledge about the reproductive biology in this species under captivity aiming to conservation. In this framework, the aim of this study was to evaluate the influence of agonistic behaviour of *S. parahybae* on reproductive performance in captivity reared females. Broodstocks that were able to spawn were selected in the pounds at the Hydrobiology and Aquaculture Unit of CESP (*Companhia Energética de São Paulo*, Southeast, Brazil) during the reproductive period (February), and then immediately induced to reproduce by combining whole acetone-dried carp pituitary extract and human chorionic gonadotropin (conventional protocol). After hormone administration until spawning, two female’s broodstocks were placed together into glass aquarium in the CESP laboratory to facilitate the observation of reproductive behavior during artificial induction. All broodstocks (n=10, 100% survival) induced to spawning responded positively to the artificial reproduction. Following the second hormonal dose, it was possible to observe a gradual increase in the animals’ activity, including aggressive behavior that even hurt one of the females. One of the females chasing the other, trapping it in a corner of the aquarium, and sometimes, these chasing were with physical contact between females, showing some bites and tail hit behavior. Later, at the end of the period artificial spawning, at the time of manually stripping for gamete collection; some females were damaged and others did not have any injury. We defined as “dominant” female, the ones which showed more aggressive behavior and did not have any injury and “no-dominant” the females that were injured. These injuries were observed throughout the body of non-dominant female, including the dorsal part of the body and caudal fin (Fig.1). All major reproductive results are summarized in Table 1. Our results contribute to improve the protocol to obtain a better reproductive performance in captivity, since their endangered status deserves special attention and urgent action for contribution to the knowledge of reproductive physiology, which is the basic premise for the program of fish restocking in the Paraíba do Sul River Basin.

Table 1

| Experimental group           | Total length (cm)                                   | Total weight (g) | Total stripped dry oocytes |
|------------------------------|---|------------------|----------------------------|
| Female (Dominant)            | 39.43 ± 0.41  | 576.67 ± 29.20   | 30.8                       |
| Female (Non-Dominant)        | 38.40 ± 0.59  | 551.67 ± 28.91   | 19.4                       |
| Total fertilization rate (%) | 21.50 ± 10.0 (Dominant); 11.00 ± 5.6 (Non-Dominant) |                  |                            |

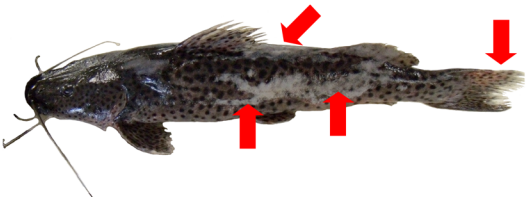


Figure 1: Non-Dominant female of *S. parahybae*, which showed injuries, including lateral and dorsal part of the body and caudal fin (arrows).

## GENOME-WIDE ASSOCIATION STUDY OF SOYBEAN MEAL TOLERANCE IN ATLANTIC SALMON

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The utilization of Fish Meal (FM) as a protein source in the Aquaculture industry has been shown not to be sustainable. Soybean meal (SBM) stands as viable replacement for fishmeal in aquaculture because of the relatively high protein content, amino acid profile and digestibility, as well as its global availability and low cost. However, SBM inclusion in salmon feed is hindered by poor performance and negative physiological impacts (e.g. enteritis). Studies in trout have shown that there is significant individual and family variation in the ability to use SBM derived protein in salmonids, and that this trait has moderately high heritability. Therefore, there is potential for the selection of lineages with higher tolerance to SBM inclusion. Marker assisted selection, (MAS) using genetic markers such as single nucleotide polymorphisms (SNPs) associated with traits of interest, can be used to compliment traditional breeding and reduce the time required to achieve genetic gains.

Fin samples from families presenting poor and good growth when fed a high SBM diet were selected and DNA was extracted from fins. Genotyping-by-Sequencing (GBS) was used to obtain genotypes for a total of 291 animals. In total, over 90,000 putative markers were identified, and 46,333 SNPs were selected for GWAS analysis (MAFs > 0.01 and rate of genotyping > 85%). Missing data was imputed using the LDKNNimp algorithm was implemented in the TASSEL v5 pipeline. Association analysis was performed using a weighted mixed linear model with kinship (measured by centered IBS) and population structure (measured by PCA). The thermal coefficient growth (TGC) data was fitted to the model and the effects of each SNPs estimated one by one using both a dominant and an additive model. The Bonferroni approach was used to control for family-wise error (FWER) (i.e. the obtained p-value was divided by the number of tests performed –  $0.05/46,333$  – p-value cutoff of  $1.08 \times 10^{-6}$ ).

Using this approach, seven SNPs (in 5 different chromosomes) were identified as being significantly correlated with TGC after FWER correction. These markers explain had an average R-squared of 0.09 and explain roughly 9% of the estimated heritability of TGC of Atlantic salmon when fed diets containing high levels of soybean meal. The markers, if validates, can be added to breeding programs and facilitate higher inclusions of soybean meal in commercial diets for Atlantic salmon.



## **PRACTICAL IMPLEMENTATION OF INTERNATIONAL AQUACULTURE BIOSECURITY PRINCIPLES**

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Guarantees guarding against the introduction of unwanted diseases are an essential element of international trade in aquatic animals. Where a country has insufficient surveillance data to prove absence of a disease, the concept of zoning and compartmentalization allows a country's competent authority to provide internationally accepted disease-status guarantees. In contrast to disease-free zones which are defined by geographical boundaries, a disease-free compartment is defined by management and biosecurity practices and can be applied to an individual farm or epidemiological unit.

Production of both specific pathogen free (SPF) trout ova and SPF koi fingerlings requires an auditable approach as proposed by the International Aquatic Veterinary Biosecurity Consortium (IAVBC). This includes maintaining a closed population of fish on a protected water source, adherence to strict biosecurity measures, strategic sampling for prioritized diseases at a statistically relevant confidence level at six-monthly interval, contingency planning that may necessitate eradication of known infected stock, and identification and remediation of critical control points to eliminate or minimize the risk of reintroducing disease. After a two-year period of surveillance with negative results, a Government endorsed certificate of veterinary inspection can provide the guarantees required for sale and export of SPF fish or ova.

Rainbow and brown trout were introduced to South Africa through the importation of eyed ova since the early colonial days, and a viable salmonid aquaculture industry developed. Far removed from salmonid producing areas in the Northern Hemisphere, South Africa remained isolated from many salmonid diseases. Following the establishment of guidelines on biosecurity and disease surveillance by the World Organization for Animal Health (OIE) and certain individual countries, South African trout farmers started successfully exporting SPF trout ova to the Northern Hemisphere during the late 1980's. Disease-status guarantees were provided by the competent authority (State Veterinary Services) based on individual importing countries' requirements. These largely reflected the standards of the OIE and depended on the national veterinary laboratory (now the Onderstepoort Veterinary Institute) acquiring the capacity to isolate and diagnose the relevant salmonid viral and bacterial diseases. In addition, farms were required to commit to, and maintain, biosecurity practices appropriate to compartmentalization. Exports have exceeded 40 million eyed ova per annum and are expected to exceed 100 million in the near future.

The export from South Africa of koi herpesvirus- and rhabdovirus carpio-free koi for the ornamental fish market has provided further opportunities. Outbreaks of disease amongst koi in South Africa, including, koi herpesvirus disease (KHVD), are frequent as the result of the poorly regulated ornamental fish industry. Yet, as in the case of trout farms, strict biosecurity measures and disease surveillance, applied to compartments breeding koi enables the competent authority to meet international disease-status requirements as well as providing valuable assurances to local industry players.

## BROOD MOLA STOCKING DENSITY IN PRAWN AND CARP FARMING TO INCREASE HOUSEHOLD NUTRITION FOR RURAL FARMERS IN SOUTHWEST BANGLADESH

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Freshwater prawn (*Machrobrachium rosenbergii*) and carp (*Labeo rohita*) polyculture are widely practicing in seasonal paddy fields (*ghers*) of southwest coastal region of Bangladesh. Farmers typically sell the prawns to fetch higher price in overseas market and carps are sold in the local market as cash crop. Meanwhile family members (particularly women and children) remain malnourished from lack of complete protein, vitamins and other minerals in their diet. The present investigation proposed to help mitigate this problem by incorporating nutrient enriched small fish (*Mola*, *Amblypharyngodon mola*) for home consumption in the current prawn-carp traditional gher farming system. A study was conducted on the farms of local producers for a period of 6 months. Mola was stocked at a rate of 1, 2 and 4/m<sup>2</sup> in treatment 1, 2 and 3 respectively, with 5 replications for each treatment. Furthermore, 5 ponds were selected randomly where mola was not stocked and treated as control (treatment 4). In all the 4 treatments stocking density of prawn and carp were 2 and 0.1/m<sup>2</sup>, respectively. Production of prawn was 455.58±14.69, 462.77±15.60, 456.28±13.94 and 362.25±17.84 kg/ha and carp was 588.11±16.47, 572.19±17.28, 586.75±15.39 and 502.92±16.84 kg/ha in T1, T2, T3 and T4 respectively. Treatment 4, lacking mola had significantly lower prawn and Rohu production compared to the other treatments. Further, mola production was higher in T2 (376.21±15.34 kg/ha) and T3 (397.66±18.41 kg/ha) groups compared to T1 (298.55±11.55 kg/ha). The study showed that the addition of mola has no adverse impact on prawn-carp production system, and may in fact improve prawn and carp production. Based on these results and the costs for initial stocking of mola, it is suggested that mola brood be stocked at a density of 2 fish/m<sup>2</sup>. Considering mola require no supplementary feed inputs for their growth, have no negative impact or may improve production of prawn and carp, and are a source of nutritious food, it is highly recommended that farmers incorporate mola in prawn-carp gher polyculture systems to enhance their nutrition and potential income opportunities.

Funded by the AquaFish Innovation Lab of the United States Agency for International Development (USAID) Cooperative Agreement No. EPP-A-00-06-00012-00 and by U.S. and Host Country partners.

(Continued on next page)

Here I am giving some tables those might help you during correction

**Table 1. Experimental design of prawn, carp and mola polyculture in gher/freshwater pond farming system**

| Species     | T1                  | T2                  | T3                 | T4                 |
|-------------|---------------------|---------------------|--------------------|--------------------|
| Prawn       | 2/m <sup>2</sup>    | 2/m <sup>2</sup>    | 2/m <sup>2</sup>   | 2/m <sup>2</sup>   |
| Rohu        | 0.1/ m <sup>2</sup> | 0.1/ m <sup>2</sup> | 0.1/m <sup>2</sup> | 0.1/m <sup>2</sup> |
| Mola        | 1/m <sup>2</sup>    | 2/m <sup>2</sup>    | 4/m <sup>2</sup>   | Nil                |
| Replication | 5                   | 5                   | 5                  | 5                  |

**Table 2. Abstract**

**Production (kg/ha) of prawn, mola and rohu from July 2015 to December 2015**

| Fish  | T1 (kg)                    | T2 (kg)                    | T3 (kg)                    | T4 (kg)                    |
|-------|----------------------------|----------------------------|----------------------------|----------------------------|
| Prawn | 455.58 <sup>a</sup> ±14.69 | 462.77 <sup>a</sup> ±15.60 | 456.28 <sup>a</sup> ±13.94 | 362.25 <sup>b</sup> ±17.84 |
| Mola  | 298.55 <sup>a</sup> ±11.55 | 376.21 <sup>b</sup> ±15.34 | 397.66 <sup>b</sup> ±18.41 | Nil                        |
| Rohu  | 588.11 <sup>a</sup> ±16.47 | 572.19 <sup>a</sup> ±17.28 | 586.75 <sup>a</sup> ±15.39 | 502.92 <sup>b</sup> ±16.84 |

*Values in the same row with different superscripts are significantly different (p<0.05).*

**Table 3, Consumption of prawn, mola and rohu from July 2015 to December 2015**

| Fish  | T1 (kg)                   | T2 (kg)                   | T3 (kg)                   | T4 (kg)                   |
|-------|---------------------------|---------------------------|---------------------------|---------------------------|
| Prawn | 18.93 <sup>a</sup> ±1.1   | 13.2 <sup>b</sup> ±0.49   | 14.86 <sup>c</sup> ±0.6   | 9.98 <sup>d</sup> ±0.82   |
| Mola  | 109.22 <sup>a</sup> ±2.25 | 118.55 <sup>b</sup> ±1.64 | 124.4 <sup>c</sup> ±2.03  | Nil                       |
| Rohu  | 135.25 <sup>a</sup> ±4.01 | 125.69 <sup>a</sup> ±2.89 | 132.36 <sup>a</sup> ±2.78 | 130.67 <sup>a</sup> ±8.89 |

*Values in the same row with different superscripts are significantly different (p<0.05).*

**Table 4. Cost benefit analysis for mola brood stocking**

| Treatments | Stocking<br>Density(ind/h) | Weight<br>(kg/ha) | Buying<br>price<br>(250Tk/kg) | Production<br>(kg/ha) | Selling<br>price<br>(200Tk/kg) | profit<br>(Taka) |
|------------|----------------------------|-------------------|-------------------------------|-----------------------|--------------------------------|------------------|
|------------|----------------------------|-------------------|-------------------------------|-----------------------|--------------------------------|------------------|

# ALLEVIATION OF AFLATOXIN B<sub>1</sub> (AFB<sub>1</sub>) TOXICITY BY CALCIUM BENTONITE CLAY: EFFECTS ON GROWTH PERFORMANCE, CONDITION INDICES AND BIOACCUMULATION OF AFB<sub>1</sub> RESIDUES IN NILE TILAPIA (*Oreochromis niloticus*)

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Research has shown some success using clay-based binders to adsorb aflatoxin in terrestrial animal feeds; however, no adsorbent has been approved for the prevention or treatment of aflatoxicosis. In the present study, growth performance, condition indices, proximate composition and protein retention efficiency of Nile tilapia were evaluated along with analysis of aflatoxin-B<sub>1</sub> residuals in muscle tissues of fish fed diets containing AFB<sub>1</sub> at 2 and 4 ppm along with either 0.5 or 1.0% calcium bentonite clay (4TX) for a period of 10 weeks. To compare the efficacy of inclusion type of 4TX clay in the diet, it was added as either a dry powder mix (PM) or water dispersed (WD) form. Inclusion of AFB<sub>1</sub> at concentrations of 2 and 4 ppm significantly ( $p < 0.05$ ) reduced weight gain, feed efficiency ratio (FER), hepatosomatic index (HSI), muscle ratio, whole-body crude lipid and protein retention efficiency (PRE), regardless of the inclusion of 4TX in the diets. However, addition of 4TX as PM in the diet improved some of those fish responses. In particular, at the 2 ppm AFB<sub>1</sub> level, 1.0% 4TX PM tended to improve % weight gain (831%) as compared to 1.0% 4TX WD (707%) or 0.5% 4TX WD (708%). Also there was a significant difference in HSI when contrasted between basal vs clay PM ( $P < 0.001$ ) and dispersion type, WD vs PM ( $P = 0.022$ ). Whole-body % moisture ( $P = 0.036$ ) and crude lipid ( $P = 0.014$ ) also were significantly affected by AFB<sub>1</sub> in diets at both 2 and 4 mg/kg. Contrast between basal vs clay WD also showed a significant difference ( $P = 0.046$ ) in whole-body crude lipid content. The AFB<sub>1</sub> residues in muscle tissues were only detected in fish exposed to the highest inclusion level of AFB<sub>1</sub> (4 ppm). Addition of 4TX in the diets significantly reduced bioaccumulation of AFB<sub>1</sub> in muscle of Nile tilapia, especially when included in PM ( $0.43 \pm 0.03$  ng/g) compared to WD ( $1.87 \pm 1.32$  ng/g) form. Thus, greater efficacy of 4TX clay was observed when included as PM compared to WD in alleviating AFB<sub>1</sub> induced toxicity in tilapia.

## EFFECT OF INCLUSION TYPE OF CALCIUM BENTONITE CLAY ON GROWTH PERFORMANCE AND BODY COMPOSITION OF NILE TILAPIA (*Oreochromis niloticus*) FED AFLATOXIN-B<sub>1</sub> SUPPLEMENTED DIETS

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The present study is planned to evaluate the protective efficacy of including particular calcium bentonite clay as a dry and wet application in the presence of dietary aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) on the health performance of Nile tilapia over a period of 10 weeks. Addition of AFB<sub>1</sub> at concentrations of 2 and 4 mg/kg significantly ( $p < 0.05$ ) reduced average weight gain (AWG), average daily gain (ADG), specific growth rate (SGR), survival rate (SR), feed efficiency ratio (FER), crude protein (CP) and crude lipid (CL), regardless the inclusion of the 4TX clay in the diets. Inclusion of 4TX clay in the diets in both dry (DF) and wet (WF) form, improved the growth performance of the fish. At 2 mg/kg AFB<sub>1</sub> level, 1.0% 4TX DF significantly increased the average weight gain ( $39.70 \pm 8.22$ g) and feed efficiency ratio ( $0.677 \pm 0.06$ ) when compared to 1.0% 4TX WF ( $34.14 \pm 5.20$ g) and ( $0.599 \pm 0.09$ ), respectively. Similarly at 4 mg/kg AFB<sub>1</sub> level, 1.0% 4TX DF significantly increased the average weight gain ( $33.19 \pm 10.24$ g) and feed efficiency ratio ( $0.640 \pm 0.03$ ) when compared to 1.0% 4TX WF ( $31.51 \pm 3.87$ g) and ( $0.618 \pm 0.02$ ), respectively. Fish exposed to 1.0% 4TX DF showed higher protein percentage and lower moisture contents when compared to 1.0% 4TX WF group. This showed the efficacy of 4TX clay in DF inclusion type over WF in reducing the AFB<sub>1</sub> induced toxicity in tilapia. It is concluded that 4TX clay positively alleviated the AFB<sub>1</sub> toxicity in tilapia especially in dry form (DF).

## MARKETING STRATEGY OF FARMED FISH IN CENTRAL UGANDA

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Aquaculture sector in Uganda currently contributes a small proportion (16%) to the total fish supply. Due to increased demand for fish both domestically and internationally and in the face of dwindling supplies from the lakes and rivers, interest in aquaculture industry has heightened due to a shift from demand for home consumption to becoming a commercial enterprise. Thus, the industry has become increasingly market-driven and therefore the linkage between production and consumption is gaining interest to policy makers. As commercial fish producers, they need to be equipped with the right information in order to make strategic decisions aimed at profit maximization. However, there is limited research work carried out in the country to inform the policy. The paper looks at marketing and pricing strategies, fish farmers use to sell their fish while remaining competitive.

The data used in this study were collected from a sample of 126 commercial fish farmers in 2015. The sample was randomly drawn from a list of fish traders/farmers generated at the district level of Wakiso, Mpigi and Mukono, in Central Region of Uganda. Data collected were cleaned, coded, and entered using SPSS spreadsheet package. The data were analyzed using descriptive statistics and econometric methods.

The results show that the respondents had a mean age of 49 years with a household size of 5.3 persons. Most of them had attained an education level of 12.2 years, implying completing O level education. Furthermore, the respondents had adequate experience of 7 years in fish farming/trade. The results also showed that majority (93.7%) of the respondents interviewed sold fish, an indication of high level of commercialization. The majority (64%) of traders practiced personal (individual) selling while 29.3% sold in a group. Those who sold individually cited the following reasons; lack of competition, convenience and opportunity to bargain with buyers. No product branding, sales promotion nor packaging was practiced. The primary sources of price and market information for the fish farmers/traders were fellow farmers (36%) followed by market traders (23%).and fisheries extension workers (16%). In terms of pricing strategies, the majority of farmers adopted single pricing strategy as a way to minimize losses. This strategy was followed by price penetration in cases where the respondent was more commercialized and had the requisite infrastructure. This followed by Cost-plus pricing.

Based on the results, they point to the need for fish farmers/traders to not solely rely on single pricing, but adopting multiple pricing and marketing strategies which would enable them take advantage of niche markets. This can only be possible when they have easy access to capital to invest in the required infrastructure such as the iced vehicles and producing required fish size demanded by the market.

## **EVALUATION OF GROWTH PERFORMANCE AND CARCASS COMPOSITION OF *Clarias gariepinus* FED WITH CASSAVA PEEL MEAL (*Manihot Esculenta*)**

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The paradigm shift of using non conventional protein supplements of plants in practical fish diets for sustainable economic growth has been a current focus in Nigeria. The present study was therefore carried out to determine the growth performance and Carcas composition of *Clarias gariepinus* juveniles fed with diets containing Cassava peel (*Manihot esculenta*). Six weeks old *Clarias gariepinus* juveniles were obtained and fed with diets containing Cassava peel inclusions. The diets contained inclusions of Maize (M) and Cassava Peel (CP) in the following proportions: Treatment I was the control fed with Diet 1 (0% CP + 35% M); treatment II fed with Diet 2 containing 25% CP + 75% M; treatment III was fed with Diet 3 containing 50% CP + 50% M; treatment IV fed with Diet 4 with 75% CP + 25%M, and, treatment V was fed with Diet 5 which was made up of 100% CP. Of all the treatments, treatment I had the highest mean value of initial weight ( $15.35 \pm 2.12$ ), while treatment II had the least mean value of initial weight ( $8.17 \pm 1.26$ ). However, both treatment I and II which are significantly different ( $P > 0.05$ ) from one another are also significantly different ( $P < 0.05$ ) from all other treatments. As for the final weight, although treatment I had the highest mean weight ( $21.28 \pm 3.06$ ). However, this observation is not significantly different ( $P > 0.05$ ) with mean final weight of treatment V ( $19.79 \pm 2.01$ ). The mean weight gain varies among the treatments. Treatment V has the highest mean weight gain ( $8.82 \pm 0.67$ ), which is not significantly different ( $P > 0.05$ ) from treatment II ( $8.08 \pm 1.03$ ), but significantly different ( $P < 0.05$ ) from all other treatments. The percentage weight gains varies considerable among the treatments, treatment II had the highest percentage weight gain (107%) while treatment IV had the least percentage weight gain. In conclusion, the observations in the present study revealed that Cassava peel meal has great potentials to replace the expensive conventional fish meal in fish diet. The study further revealed that fish subjected to these diets will be nutritional upright and therefore, can be consumed conveniently by the populace.



## CARCASS QUALITY OF SOME COMMONLY CONSUMED FISH IN MINNA, NIGER STATE, NIGERIA

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Study on the carcass quality of some commonly consumed fish in Minna Metropolis, Niger State, Nigeria was carried out between May 2016 and August 2016. Fish samples were purchased at Kure Ultra-Modern market and Mobile fish market. Proximate analysis (AOAC 2000) of fish samples showed highest moisture contents in *Tilapia* (76.61%) and lowest in *Clarias* (74.77%), which did not differ significantly ( $P>0.05$ ). *Heterotis* species had the highest ash (3.06%) and protein contents (13.34%), while *Clarias* had the highest oil extract, which differ significantly ( $P<0.05$ ). There was variations in nutritional elements in the months:- *Heterotis* had highest ash contents in May (3.06%), June (1.88%) and July (1.95%) and *Clarias* (1.46%) in August. *Clarias* also had highest oil extract in May, June, July and August. Moisture contents, oil extracts of fish samples did not differ significantly ( $P>0.05$ ) between the markets. Moisture contents and oil extracts were higher in samples at Mobil fish market, while ash contents and crude protein were higher in samples obtained from Kure ultra-modern market. *Heterotis* had the highest level of mineral compositions with the exception of Na. There were variations in the mineral compositions of fish samples in months. Na was highest in May, June and July in *Clarias*, which differ significantly ( $P<0.05$ ), Ca was highest in May, July and August in *Heterotis*. While Ca and P were highest in both markets for *Heterotis*, Na, Mg, Fe and K varied between fish samples in these markets. Sensory evaluation parameters showed significant difference ( $P<0.05$ ) in colour, texture, appearance, taste and aroma of fish samples. *Tilapia* had the best evaluation parameters except texture. There was also significant difference ( $P<0.05$ ) of these parameters in month between samples. Although *Clarias* had the highest nutritional composition, all samples are recommended for consumption with *Clarias* the most preferred.

## STUDY OF HEAVY METAL CONCENTRATION IN WATER AND FISH SPECIES IN RIVER KUNKO, DABBAN, NIGER STATE, NIGERIA

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Bioaccumulation of toxic heavy metals to man through aquatic life occurs through industrial, runoff, agricultural, municipal and urban waste. Heavy metals such as lead (Pb), iron (Fe), manganese (Mn) chromium (Cr) and Zinc (Zn) have National environmental standard regulation and enforcement agency (NESREA) limits, above which they are toxic. Some of the toxic effects include:- cancer, damage to the nervous system, reduced growth and development, and in extreme cases lead to death. River Kunko, has its source in a densely populated area which has agricultural activities like banana plantations and domestic activities. Some of the tributaries that flow into the River contain various potential hazardous heavy metals. In this study, concentration of these heavy metals (Zn, Cr, Pb, Fe, Cu and Mn) in both water and fish samples were documented. The fish species used in this study include: *Chromidotilapia guntheri guntheri*, *Sardnella albella*, *Clarias garienpinus*, *Clarias lazera* and *Ctenopharyngodon idella*. Five different water sampling stations used in this study include In water different five sampling stations were also used in this study include: Kunko Nnayagbi, Kunko Langifu, Kunko Zukomintsun, Kunko Tako, and Kunko Alhaji Abu. The samples were analysed using Atomic Absorption Spectrophotometer (AAS). The mean result (mg/l) for water ranges from: Pb (0.000-0.051), Fe (1.105-24.115), Mn (0.091- 0.425), Zn (0.040-0.424), Cr (0.000-0.740), and Cu (0.089-0.254). And fish species mean result (mg/kg) ranged from Pb (0.000-0.031), Fe (1.050-14.545), Mn (0.020-3.250), Zn (0.040-12.870), Cr (0.000-8.320) and Cu (0.000-0.740) respectively. Although, *Clarias garienpinus* showed significant higher bioaccumulation of heavy metals ( $P < 0.05$ ) than other fish species. The increase concentration of heavy metals in fish species in this study indicates that they have high ability to retain heavy metals than the water samples.

## HEAVY METAL CONCENTRATION IN WATER AND FISH SPECIES IN RIVER KUNKO, DABBAN, NIGER STATE, NIGERIA

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## **IDENTIFICATION OF THE SUITABLE CRITERIA FOR TAGGING OF NILE TILAPIA (*Oreochromis niloticus*) FINGERLINGS WITH PIT TAGS AND ITS EFFECT ON SURVIVAL, GROWTH AND TAG LOSS**

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Individual fish tagging is an essential process for many biological investigations such as fish tracking/monitoring in fish migration and genetic improvement programs and it is often done in early life stages of fish while it may have an adverse effect on fish growth and/or survival. This study was conducted in order to identify the criterion (age, weight or length) that can be used to judge the success of tagging tilapia fingerlings at its smallest possible size and to study the effect of tagging on growth and survival. A total number of 1550 tilapia fingerlings were used in this study. The fingerlings were of varied ages (25-64 days), individual weights (1.5-20 g) and total lengths (3.9-9.8 cm). Fish were split into two groups, tagged group of 767 fingerlings tagged with passive integrated transponders (PIT) tags and non-tagged group of 732 fingerlings. All fish were individually weighed at the time of tagging and ten days after tagging, there were no significant differences in fish weight between tagged and non-tagged fish. Results from the tagged group showed that 657 fingerlings (86%) of tagged fish survived after tagging, 85 (11%) died and 25 (3%) lost their tags while they were still alive. Receiver operating characteristic (ROC) curve analysis was used to evaluate the discrimination threshold of age, weight, length and condition factor that best discriminates between live and died fish. Discrimination thresholds were 51 days, 4.5 grams, 5.6 cm and 1.9 for age, weight, length and condition factor (K) respectively, with significant differences ( $P < 0.0001$ ) for weight and length and not significant ( $P > 0.05$ ) for age and K. Eighty one percent of dead fish (69 fish) happened when fish was tagged at less than 4.5 grams and 73% (62 fish) of the dead fish coincided with tagging fish of less than 5.6 cm. Current results demonstrates that both weight and length could be used as principle criteria to determine the expected success of tag retention with minimal negative impact on growth and survival of the tagged fish.

## **EXPLORING CLIMATE-RESILIENT ADAPTATIONS OF FARMED FISH FOR CLIMATE-SMART AQUACULTURE IN AFRICA**

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Exploring climate-resilient adaptations in farmed fish is one strategy for helping improve food security for the world's growing population under changing environmental conditions. People rely on fish as a primary source of protein and income, supporting a rapidly growing aquaculture industry that provides roughly half of the global fish supply. In an era of climate change and high demand for animal protein, increasing the production of fish through sustainable and environmentally sensitive practices is critical. The development of climate-smart aquaculture, which incorporates practices that improve climate resilience and mitigate negative environmental impacts, can provide responsible management strategies to the aquaculture industry. One aspect of this effort involves optimizing the culture of fish species that are adapted for coping with the predicted impacts of climate change, including a tolerance for hyposaline conditions, wide temperature ranges, and/or the ability to breathe air. AquaFish Innovation Lab's work seeks to take advantage of the evolutionary ecology of these species in their natural environments to create more opportunities for climate-smart aquaculture. AquaFish efforts include incorporating the sustainable culture of air-breathing species into the fish farming industry and identifying climate-resilient tilapia strains. A major part of this work requires that adverse impacts are mitigated, therefore, AquaFish researchers investigate the wide range of positive and negative tradeoffs associated with fish production to ensure that practices remain environmentally and socially responsible.

## PATHOPHYSIOLOGICAL EFFECT OF AQUEOUS EXTRACT OF *Moringa oleifera* LEAVES ON *Clarias gariepinus* CHALLENGED WITH *Ichthyophthirius multifiliis*

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*Ichthyophthirius multifiliis* (Ich), a dangerous ectoparasite causes white spot disease and economic losses in fish farms. A majority of the chemotherapeutic agents of ichthyophthiriasis are either pollutants of the environment, banned or lack efficacy. The present work was focused on the evaluating different immersion treatments of ichthyophthiriasis of African catfish (*Clarias gariepinus*) with non-chemical and environmental friendly parasiticides, *Moringa oleifera*. A total of 800 healthy parasites-free post juvenile catfish were disinfected with 0.5% potassium permanganate. They were challenged with about 44,000 theronts obtained through serial passages by cohabitation. Seven groups (A-G) of post juvenile fish were used in the experiment in randomized complete block design with groups A, B and C as the uninfected control, Ich-infested not treated and Ich-infested treated with standard drug (fish cure) respectively, whereas, the groups D-G exposed to aqueous leaf extract of *M. oleifera* (LEMO) in dip (1,500 mg/L - 4,500 mg/L for 1h), short term (150 mg/L – 450mg/L for 24h – 96h) and prolonged bath treatment (15 mg/L – 45 mg/L for 0 – 15days). The adult parasite (trophont) burdens on the experimental fish were determined in one time point, two time points and four time points. Gross examinations, histopathological alterations, hematological parameters, antioxidants enzymes and biochemical parameters were determined using standard protocols. Data obtained were subjected to analysis of variance at 5% probability while differences between means were partitioned using the Duncan New Multiple. The results showed almost complete elimination of the trophonts both in the gills and the bodysmear of *Ichthyophthirius multifiliis* infested fish. The observed curative potential of the various extract-treated groups and standard drug-treated groups were all significantly different from the infected non-treated group ( $p < 0.05$ ). The knockout efficacy in short term bath treatment was significantly different ( $p < 0.05$ ) from the control. The ich-infested fish had visible lesions and histopathological alterations in the gills and skin among the groups. Parked cell volume, haemoglobin, white blood cell, red blood cell, differential count and the blood bicarbonate, chloride, potassium, sodium, had significant ( $p < 0.05$ ) changes among the experimental groups. Some of the observed changes during the immersion treatments did not differ significantly with the Fish cure treatment ( $p > 0.05$ ). The antioxidant parameters (catalase, superoxide dismutase, glutathione peroxidase and lipid peroxidations in the gills and muscles of ich-infested fish among the groups) and biochemical parameters (total protein, albumin, globulin, urea, creatinine, lactate dehydrogenase, aspartate, alanine aminotransferase) C-reactive protein showed significant changes ( $p < 0.05$ ) among the ich infested fish. However, *Moringa oleifera* has proved to be an effective non chemotherapeutant in controlling the skin-parasitic ciliate, *Ichthyophthirius multifiliis* of *Clarias gariepinus*.

## **GENDER ROLE IN THE CONSTRUCTION AND OPERATION OF TRADITIONAL FISHING GEARS IN ANAMBRA STATE NIGERIA**

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The gender role in the construction and operation of traditional fishing gears in Anambra state was studied in three fishing communities; Otuocha, Ogbaru and Ebenebe. Primary data was obtained from samples drawn from the population of fisher folk in the selected communities by simple random sampling through personal interview and structured questionnaires. Results showed that 58.8% of the respondents are males engaging in fish gear production and operation. 41.7% are females. 86.4% are females engaging in fish processing while 13.6% are males. 83.9% are females engaging in fish trading while 16.1% are males. 86.7% of the respondents source their funds through personal savings. Significantly more males engage in fish gear construction and operation than females, while more females engage in fish trading than males. The study revealed the following challenges; lack of access to credit, lack of access to gear materials, high cost of gear materials, lack of formal training, tedious nature of the occupation, lack of storage facilities, lack of standardized markets and insufficient canoes and boats.

## **CRITICAL SUCCESSION FACTORS OF CATFISH FARMING IN ANAMBRA STATE NIGERIA**

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The critical success factors of catfish farming in Anambra state were studied to identify the major challenges faced by catfish farmers. Structured questionnaires were used to collect relevant data from a total of two hundred and eighty respondents selected by stratified random sampling technique from the twenty one local government areas of Anambra state. Descriptive statistics and five point likert scale are the analytical tools used. It was observed that the critical factors include; lack of capital, poor quality fish seed, scarcity of fingerlings, high cost of fish feed, lack of extension services, epileptic power supply, inadequate record keeping and lack of sufficient knowledge in catfish farming operations. Efforts to overcome these challenges will place catfish farming on the right pedestal for sustainable catfish farming in Anambra State Nigeria.



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# HISTOPATHOLOGICAL EFFECTS OF PARAQUAT DICHLORIDE ON THE KIDNEY, LIVER, GILLS AND TISSUES OF TILAPIA *Oreochromis niloticus* FINGERLINGS

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Paraquat, a non-selecting organic herbicide is used to control weeds in areas where labour is in short supply and finds its ways to aquatic systems through run-offs or its usage in integrated fish culture systems. It is quick acting and care must be taken during application. This study investigates the effects of varying concentration of the herbicide on the water quality parameters and on the kidney, gills, liver and tissue of *O. niloticus* fingerlings.

Fingerlings of average length and weight of 4.2cm and 0.4g were gotten from the Departmental fish farm and stocked randomly at 2L<sup>-1</sup> in 43 x 20cm plastic aquaria. They were acclimatized for a period of two weeks and fed twice daily at 3% body weight with 40% CP diets with feeding stopped 24 hours before commencement of bioassay. The fish were exposed to varying concentrations of herbicide at 0mgL<sup>-1</sup>(control), 3.33mgL<sup>-1</sup>, 6.66mgL<sup>-1</sup>, 10mgL<sup>-1</sup> and 13.3mgL<sup>-1</sup> (table 1). The Lethal concentration (LC<sub>50</sub>) after 96 hrs exposure was 4.80mg/L. The mean daily water quality parameters analysed were temperature 26 ± 0.12°C; Dissolved oxygen 6.2 ± 0.16 mg/L, pH 6.8 ± 0.11, Conductivity 176 ± 0.18 us/cm, COD 24.5 ± 0.19 mg/L and Alkalinity 33.1 ± 0.17 mg/L. Analysis of Variance observed significant differences (P<0.05) between Dissolved oxygen, COD and pH between the various concentrations and significant differences (P<0.05) in Conductivity, COD and Alkalinity between the concentrations and the control.

The Histopathological results of the exposed fish showed erosions, lesion, necrosis, malignancy, pigment and inclusion bodies in the gills, periportal hepatic degeneration, necrosis and periportal connective tissue was very prominent in the liver, severe diffuse tubular necrosis in the kidney and no visible lesions in the tissues. Varying the concentration of the toxicant resulted to respiratory stress, sudden quick movements and erratic swimming. Mortality was identified by absence of movement in fish and it increased with increase in concentration of herbicide (table 1). Dead fish were removed immediately.

Paraquat dichloride is toxic to *O. niloticus* and its usage on farms should be in the recommended dosage most especially in integrated fish culture systems.

Table 1: Mortality and Probits of concentration of Paraquat dichloride

| Mortality (%) | Probits Units | -Probability | Concentration (mg/l) | Log of concentration |
|---------------|---------------|--------------|----------------------|----------------------|
| 0             | -             | -            | 0                    | -                    |
| 10            | 3.72          |              | 3.33                 | 1.20                 |
| 90            | 6.28          |              | 6.67                 | 1.90                 |
| 100           | 7.37          |              | 10.00                | 2.30                 |
| 100           | 7.37          |              | 13.30                | 2.59                 |

## THE DOMESTIC AQUARIUM TRADE IN INDIA IS SHIFTING FROM A SUPPLY DRIVEN TRADE TO A DEMAND DRIVEN TRADE

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The domestic aquarium trade has emerged as a fast developing economic activity in India since the beginning of 21<sup>st</sup> Century. The total value of trade has increased from 550 million INR (7.8 million US\$) to 3000 million INR (42.85 million US\$) during the last decade. It is because of increasing number of aquarium hobbyists which is 2% of a total of 50 million (2015-16) middle income group house-holds (MIGHh) in country. A high growth of domestic trade was a mixed function of increasing number of MIGHh, change in behavior of Indian buyer's to spend a part of income on hobby, easy & cheaper availability of aquarium products and promotional effort by government. It resulted in formation of a cyclic relationship between demand (number of hobbyists) and supply (aquarium retail outlets) i.e. the demand triggered the supply and vice-versa. However, the Indian aquarium hobbyist was mainly comprised of novice hobbyists and the selection of fish, size of aquarium and accessories to be installed were mainly decided on the advice of aquarium trader depending on budget. The availability of varieties of fish and aquarium accessories were a major limiting factor. The low value fish (Table-1) were in high demand owing to supply and limited knowledge of novice hobbyists. A novice hobbyist has now grown to a matured hobbyist through experience and easy accessibility of information. The low value fish now constitutes only 60% of the total traded fish whereas medium, high and premium value fish contributes 31%, 7% and 2% respectively. The trend is further expected to shift towards high and premium value fish with the projected GDP of 8-10% and number of MIGHh to reach 113.80 million by 2025-26. The domestic aquarium trade is projected to achieve a value of 12000 million INR (171.42 million US\$) in next 5-6 years.

**Table-1 Value\* based categorization of ornamental fish produced and/or traded in Indian domestic market**

| <b>CATEGORY **</b>   | <b>NAME OF VARIETIES (Common Name)</b>  |
|--|---|
| Low Value Fish<br>INR: 15-50 each<br>US\$: 0.20-0.70 each            | Gold, Koi, Sharks (tiger, albino), Gouramy (blue, kissing, golden), Angels (black, tiger, golden), Barbs (rosy, tiger, tin foil), Tetra (B.A., lemon, widow, serpae), Live bearers, Fighters etc.   |
| Medium Value Fish<br>INR: 50-200 each<br>US\$: 0.70-3.00 each        | Gold, Koi, Sharks (silver, red tail, rainbow), Gouramy (pearl, giant), Angels (platinum, diamond, koi), Barbs (filamentosa, danisonii, ticto), Tetra (neon, cardinal, rummynose), Live bearers, Fighters, Oscars, Silver dollars, Fire mouth, Shevrum, Feather fish, shrimps etc. |
| High Value Fish#<br>INR: 200-2000 each<br>US\$: 3.00-28.00 each      | Gold, Koi, Oscars, Silver dollar, Parrot, Texas, Flower Horns, Varieties of Discus, Red devil, Green terror, Lobsters, Several other varieties of chichlids, Marine ornamental fish etc.  |
| Premium Value Fish<br>INR: Above 2000 each<br>US\$: Above 28.00 each | Flower Horn, Discuss, Arowana, Several other varieties of chichlids, Marine ornamental fish etc.  |

\* Maximum retail price, \*\* A numbers of fish varieties are grouped in more than one category depending on size.

# Many of the high & premium value exotic varieties are now bred locally in country.

## **THE TILAPIA SECTOR IN SOUTHERN AFRICA: STATUS AND INTERACTION WITH GOVERNMENTS**

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In contrast to much of Asia and North Africa, Southern Africa has a poorly developed tilapia sector with only a few large scale commercial participants. Regional variation is also marked with Zambia, Mocambique and Botswana moving ahead with several large scale projects, and South Africa falling ever further behind. Zambia now boasts some of the continent's most successful operators with not only the well-known Lake Harvest cage farming operation, recently relocated from Zimbabwe to Zambia, but also other privately owned commercial pond projects in diverse parts of the country that are now coming on-stream and putting significant tonnages of fresh fish into the market. As in Zambia, Mocambique and Botswana also have active cooperation between government agencies and tilapia aquaculture promoters.

In South Africa, despite the claim of government support for aquaculture both at a commercial as well as food-security level through Operation Phakisa, any serious development is virtually stillborn through bureaucratic and regulatory burdens imposed on the industry. Conservation agencies, which effectively have the veto on any project, spend much time regulating the struggling sector, ignoring the upriver invasion of alien tilapia from neighbouring countries, yet making little to no attempt to establish sanctuaries for threatened tilapia species. Already smothering legislation is compounded by an unnecessary but threatened Aquaculture Bill that removes aquaculture from the norms and standards of agriculture and imposes on it yet another layer of regulatory burden. An obsession with establishing Aquaculture Development Zones takes no cognisance of why those existing projects in such zones have already failed, and further threatens private aquaculture development. Such myopic policies fail to recognise how aquaculture has managed to thrive in other countries such as Egypt, Zambia and Thailand.

## POND TILAPIA AQUACULTURE SUCCESSES IN SOUTHERN AFRICA

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The successes of Asian aquaculture are, up to recently, inexplicably copied with reluctance in Southern Africa, where reinvention of the wheel of failure is regrettably a frequent event. Government projects using poor quality, mixed sex tilapia with production methodology dating back to the 1960s are still the norm rather than the exception. However, innovative private farms in Zambia, Zimbabwe, Botswana and Mozambique are now taking the lead.

Zambia in particular now boasts several large scale pond farms with significant monthly tilapia production. Kafue Fisheries near Lusaka has for some time been putting 100 tons a month into the market, in successful competition with imported Chinese tilapia. Combination pig and tilapia rearing methodology is clearly successful. More recently new pond farms near Livingstone are achieving yields of 17.5 tons/ha with carefully made ponds, and precision management, using a rearing regime of chemical fertilisation and feeding. Each pond harvested represents 17.5 tons of fish no longer netted from the Zambezi river: surely a conservation plus.

Use of indigenous species is not always necessary to protect the environment from alien tilapia invasion. New techniques coupled with structures to prevent escapes and a policy of no water returned to natural water-bodies safeguards against possible alien species invasion.

In Botswana, use of *Oreochromis andersonii*... surely the tilapia species with most untapped aquaculture potential... is replacing the unsustainable gill-net fishery with either advanced RAS technology, and more recently, pond-rearing using the Zambian model. Production of *O. andersonii* under cooler winter climatic regimes than preferred by *O. niloticus* holds much promise.

## INTESTINAL MICROBIOTA CHANGES IN LAMBARIS FED PROBIOTIC

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This study aimed to evaluate the probiotic (*Lactobacillus* spp.) effect on yellow tail lambari (*Astyanax bimaculatus*) microbiota and gut morphology. For this, eighty fish were distributed in ten tanks equipped with a recirculation system. The experimental units were divided into two treatments: fish fed with *Lactobacillus* spp. supplement and fish fed an unsupplemented diet (control). Following 24 h of starvation at the end of the experimental period (30 days), guts of three fish from each tank were removed and pooled to microbiological and histological analysis. The pooled fish guts were homogenized and serially diluted 1:10 in 0.65% of NaCl sterile saline. Samples from each dilution were cultured in PCA, TCBS agar, cetrimide agar, and MRS agar media and incubated for 48 h at 30°C. Samples from the anterior region of intestine were collected and fixed in FBS 10% during 48h. After fixation, the samples were washed and dehydrated to be embedded in paraffin. Sections 5  $\mu$ m thick were stained with hematoxylin and eosin, and photomicrographs were made using an Epifluorescent microscope equipped with Image Q Capture Pro 5.1 software. From the images, it was possible to measure the length, width and perimeter of the villi ( $\mu$ m). Microbiological data were  $\log_{(x+1)}$  transformed, and all data were assessed by Student's -t test. All tests were conducted at a 5% level of significance.

Lambaris fed a probiotic diet had a lower count of *Vibrios*, *Pseudomonas* and *Staphylococcus* and a higher count of LAB when compared to control treatment. The concentration of viable culturable heterotrophic bacteria did not differ between treatments (Table 1). In light microscopy, the probiotic group showed morphological differences in length, width and perimeter of the villi compared to the unsupplemented group (Table 2).

In conclusion, the probiotic (*Lactobacillus* spp.) changed positively the microbiota and morphology gut of yellow tail lambari (*A. bimaculatus*).

**Table 1:** Bacterial counts ( $\log_{(x+1)}$ ) in the gut of yellow tail lambari (*A. bimaculatus*) fed *Lactobacillus* spp. supplement (probiotic) and control (unsupplemented diet).

| Treatment        | VCHB <sup>1</sup> | <i>Staphylococcus</i> spp. | <i>Vibrios</i> spp. | <i>Pseudomonas</i> spp. | ALB <sup>2</sup> |
|------------------|-------------------|----------------------------|---------------------|-------------------------|------------------|
| Control          | 5,9 $\pm$ 0,6*    | 5,9 $\pm$ 0,0*             | 4,3 $\pm$ 0,6*      | 4,0 $\pm$ 0,2*          | 2,4 $\pm$ 0,1    |
| Probiotic        | 5,3 $\pm$ 0,5     | 4,2 $\pm$ 0,2              | 3,0 $\pm$ 0,3       | 3,3 $\pm$ 0,1           | 4,8 $\pm$ 0,7*   |
| Significance (p) | 0,046482          | 0,025621                   | 0,040078            | 0,015004                | 0,017071         |

<sup>1</sup>Viable culturable heterotrophic bacterias; <sup>2</sup>Acid Latic Bacteria

**Table 2:** Length, width and villi perimeter of yellow tail lambari (*A. bimaculatus*) fed *Lactobacillus* spp. supplement (probiotic) and control (unsupplemented diet).

| Treatment        | Length (mm)      | Width (mm)      | Perimeter (mm)    |
|------------------|------------------|-----------------|-------------------|
| Control          | 189.0 $\pm$ 6.6  | 67.6 $\pm$ 4.8  | 418.9 $\pm$ 12.4  |
| Probiotic        | 201.3 $\pm$ 3.6* | 74.9 $\pm$ 2.2* | 459.0 $\pm$ 26.0* |
| Significance (p) | 0.023701         | 0.038026        | 0.036685          |

## **GATSBY AND MSINGI: FINANCIAL MODELS FOR AQUACULTURE IN EASTERN AFRICA**

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The Gatsby Charitable Foundation is one of the UK's largest. Its Africa Programme is focused on agricultural sector development in East Africa. Gatsby is the founding partner of Msingi, whose strategy is to encourage transformative sector development in East African sectors where there is a potential comparative advantage, which could benefit from new innovation, technology transfer, capacity building and investment. Msingi's focus is on aquaculture.

In Africa generally aquaculture has not seen the scale and pace of developments observed elsewhere in the World. East Africa, with its abundance of fresh water resources has fallen behind the limited achievements of other regions in Africa. The small number of aquaculture operations, which do exist, have struggled to scale up and achieve consistent profitability. There are considerable constraints, which need to be overcome, not least access to the required inputs.

The Msingi approach is focused on addressing structural factors inhibiting growth of a successful aquaculture industry in East Africa. All the operators face the same issues with perhaps the biggest issue being access to consistently high quality fish feed at a reasonable price. Msingi is looking at this issue and starting to focus on genetics, fingerlings, upgrading local skills and engaging with third party interests and stakeholders. Msingi believes that the best way to tackle the development of a vibrant aquaculture sector in East Africa is to approach the sector with an integrated plan to address these constraints. Investing in aquaculture businesses in isolation is unlikely to provide the sector with the stimulus needed to achieve a rapid change in the growth trajectory of the sector.

This will require a significant level of investment, much of it into operations, which will initially be loss making until they achieve critical scale or operational capacity. There is no shortage of available funding for businesses and projects which provide the potential for risk adjusted market returns. The shortage is of opportunities which meet the requirements of these commercial financiers. Aquaculture in East Africa has struggled to attract any private equity investment. There are no examples of demonstrable success. And this situation will continue until a new approach is taken to provide the investment stimulus the aquaculture industry requires.

The funding which is required is not likely to be provided by a traditional private equity SME fund structure. It requires a long term patient investment company able to provide sub commercial or concessionary finance as well as debt and equity. The investment company will be most effective if it can build a pipeline of opportunity. This will require some extensive supportive grant/donor funders to operate alongside and in alignment with the investment company. An efficient model would see the investment company as an associate of an established private equity business. The private equity business would provide the essential private equity disciplines, back office and administrative support and some oversight but the investment company would have its own senior management team with experienced investment and aquaculture professionals.



# ANGIOTENSIN I-CONVERTING ENZYME INHIBITORY PEPTIDES FROM AN ENZYMATIC HYDROLYSATE OF FLOUNDER FISH (*Paralichthys olivaceus*) MUSCLE AS A POTENT ANTI-HYPERTENSIVE AGENT

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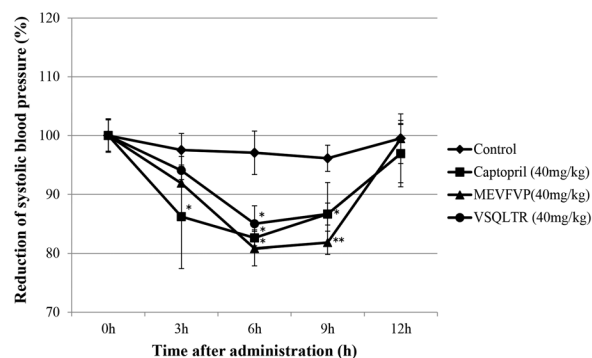
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**Background.** Fish protein hydrolysates (FPH) possess nutritional, antioxidative, anti-hypertensive, antimicrobial, and immunomodulatory properties. In particular, Olive flounder, *Paralichthys olivaceus*, is one of the most important marketed fish on Jeju Island, and major cultivated fish species occupy 98% of the domestic aquaculture market in South Korea. Olive flounder has high value as food source of protein. But, studies about bioactive peptides from flounder fish muscle (FFM) have not been reported until now. Therefore, we investigated the effects of bioactive peptide from hydrolysates of FFM on anti-hypertension activities.

**Materials and methods.** The hydrolysates were prepared by enzymatic reactions of FFM using eight commercial proteases such as papain, trypsin, kojizyme, and pepsin. Further separation of the pepsin hydrolysate with the highest biological effects was performed by ultrafiltration, gel filtration, and reverse-phase high performance liquid chromatography.

**Results.** The aim of this study was to purify peptides with anti-hypertensive properties from a hydrolysate of flounder fish muscle. Among four proteolytic hydrolysates, pepsin showed the strongest angiotensin-I converting enzyme (ACE) inhibitory activity. The pepsin hydrolysate was fractionated by ultrafiltration, gel filtration chromatography and reverse-phase high performance liquid chromatography, and two novel peptides were purified. The  $IC_{50}$  values of the two peptides were 79  $\mu$ M and 105  $\mu$ M, respectively, and the Lineweaver-Burk plots suggested that they act as a competitive and a non-competitive inhibitor of ACE, respectively. Moreover, we predicted the 3D structure of ACE and used a molecular docking program to simulate binding between ACE and the peptides. These molecular modeling results indicated strong binding and interaction energies, and systolic blood pressures were reduced by administration of both peptides in spontaneously hypertensive rats. These results suggested that the enzymatic hydrolysate of flounder fish muscle includes novel ACE inhibitory peptides that may be beneficial as a functional food for treating hypertension.

**Conclusion.** We expected an ACE-inhibitory benefit though intake of flounder fish as functional food.



**Figure 1.** Reduction of systolic blood pressure of SHR rats after the oral administration.

## TRANSCRIPTOME PROFILING OF THE GANGLION TISSUES FROM SMALL AND LARGE ABALON (*Haliotis discus hannai*) GROUPS

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Abalone (*Haliotis discus hannai*) can be considered as a member of a larger molluscan class, the gastropodas that have a high potential for commercial exploitation in Korea, Japan and China. However, there are few studies on the growth-related information of abalone, and our understanding is further limited by a lack of genomic information. In order to understand the growth response of abalone, the transcriptomic profiles of the ganglion from small and large abalone groups were obtained using NextSeq™ 500 (Illumina). An average of 70 million clean reads per library was obtained, and approximately 76.5 % of these genes were successfully mapped to the reference sequence. De novo assembly was performed to obtain a comprehensive transcriptome profile between small and large abalone groups, and produced into 275,064 genes among 355,757 transcripts (average length of 683 nucleotides, of which 72,660 could be annotated). The genes were assigned to biological processes and functions after annotation in Gene Ontology, Eukaryotic Ortholog Groups and Kyoto Encyclopedia of Genes and Genomes. Our results provide valuable information for further analysis of the mechanisms of abalone growth.

## USING *Trematocranus placodon* AS A BIOLOGICAL CONTROL AGENT OF SCHISTOSOMIASIS SNAIL HOSTS

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A breeding program for *Trematocranus placodon*, a Lake Malawi molluscivorous cichlid that can be used as a biological control agent of snails in schistosomiasis control programs, was initiated at Lilongwe University of Agriculture and Natural Resources in 2003. Initial experiments using hatchery-bred fish in snail control revealed that snails were quick to sense the presence of the predator or predation on their conspecifics in laboratory settings, which improved their protective ability. An experiment was conducted to quantify the behavioural responses of snails in the presence of cues from the predator (*T. placodon*) or killed conspecifics.

Sixty *Bulinus tropicus* snails were put in each of the nine 30-litre glass aquaria. *Bulinus tropicus* was used because it is not a host of *Schistosoma haematobium*, a parasite that causes Schistosomiasis. There were three treatments: no chemical cues were applied (treatment 1), cues from killed conspecifics (treatment 2) and cues from the predators (treatment 3). Each treatment was replicated thrice in a completely randomized design. Chemical cues from killed conspecifics were prepared by killing five snails after administering an anaesthetic and leaving them in a 1 litre beaker of water for an hour. The water was then added to Treatment 2. Chemical cues from the predator were prepared by putting five  $40.32 \pm 0.27$  grams fish in 30 litre tanks for 24 hours and removing them just before the snails were put in. The aquaria had half of the bottom covered with stones. Twenty snails were then put in each of the nine aquaria. After one hour, the number of snails that were on the open bottom, hiding under stones and outside the water was recorded.

The result showed a significant ( $p$ -value = 0.0001 ) association between application of cue and behavioural response of *Bulinus tropicus*. Cue application resulted in snails moving away from the open water. Hiding behaviour was higher when the snails sensed the cues from killed conspecifics than from the predators. We suggest that cues from conspecifics may have alarm signals to alter snail behavioural response.

**Table 1.** Hiding and crawl-out behavioural responses of *Bulinus tropicus* in 30-litre aquaria to chemical cues after one hour of cue application

| Type of cue  | Number of snails |        |             |
|--------------|------------------|--------|-------------|
|              | Open water       | Hiding | Crawled out |
| No cue       | 34               | 12     | 14          |
| Conspecifics | 11               | 37     | 12          |
| Predators    | 21               | 28     | 11          |

$\chi^2 = 24.96$ ; degrees of freedom = 4,  $p$ -value = 0.0000



**Figure 1.** *Trematocranus placodon*, Lake Malawi molluscivorous cichlid

## **WOMEN INVOLVEMENT IN COASTAL ACTIVITIES AND COMMUNITY BASED MARICULTURE IN ZANZIBAR, TANZANIA**

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Women play various roles along the coast of Zanzibar, Tanzania which contributes to their socio economic wellbeing. Usually women have traditionally been involved in gleaning the coast, collecting shells of different types as well as collection of sea cucumber, also crab harvesting and recently seaweed farming. Of late they are also actively involved in processing and selling fish and from 2003 in bivalve farming and from 2006 in half pearl farming including jewelry making using shells. Despite their involvement in activities of this sector, women's operations are often small-scale and their incomes small as compared to their men counterparts. They are also faced with various constraints. This paper elaborates the different activities women do and how they have been empowered economically through attempts to culture bivalves, half pearls and making jewellery using shells in a sustainable manner using no take zones and a new methodology of spat collectors. Also how they have become stronger through collaborative efforts in enhancing these Mari culture initiatives. The achievements, challenges obtained up to now and the future directions are presented. This case studies could be useful examples for other countries facing similar problems to try.

## STRAIN EVALUATION OF THAI ASIAN SEABASS, *Lates calcarifer* (Bloch, 1790): GROWTH AND GENETIC DIVERSITY

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Culture of Asian sea bass was well established in Thailand long before the 1970s when the Thai Department of Fisheries was the first to succeed in breeding the species. The fish is known for its rapid growth rate, attaining a size of 3-5 kg within 2-3 years. Thailand has been the top producer for both fingerlings and marketable fish for both domestic and export markets. However, the production potential of Asian sea bass is limited by a lack of genetically improved strains. In this study, we evaluated the growth performance of four strains of Asian sea bass which have been used by small- and medium-sized farms located along the east coast and the Upper Gulf of Thailand.

Samples of 90 days-post-hatch fingerlings ( $9.15 \pm 0.20$  to  $9.74 \pm 0.40$  g) of four strains (CHB, CHP, CHS and SMS) were obtained from private hatcheries. Growth performance of Asian sea bass strains was tested under two culture conditions; floating cages in the river and cages in earthen ponds. At the end of the grow-out period (approximately 220 days), all fish were harvested. Fish were measured for body weight (BW), standard length (SL) total length (TL) and body depth (BD). Yields and survival rates were calculated at harvest. To assess genetic diversity within and among populations, fin clips of 50 fish from each of the four strains were collected and genotyped at seven microsatellite loci.

Overall, fish grown in ponds were 30% heavier than those raised in floating cages when measured at harvest. The Chacheongsao (CHS) strain, which exhibited the highest average numbers of microsatellite alleles (9.4) with effective population size of 55, was the best-performing strain in both culture conditions. Final weights were 478.24 g and 730.23 g, respectively, for fish grown in cages and earthen ponds, with similar FCR (1.75 and 1.73) and survival rates (63.22 and 68.5%).

**Table 1** Least-squares means of growth traits at harvest among Asian sea bass strains and ANOVA results (*p*-values) from earthen ponds. Entries on rows with different superscripts differ significantly at  $p < 0.05$ .

|                      | Strain                    |                           |                           |                           | ANOVA   |        |
|----------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------|--------|
|                      | CHB                       | CHP                       | CHS                       | SMS                       | Strain  | Cage   |
| Phase I (90-210 d)   |                           |                           |                           |                           |         |        |
| Start weight (g)     | 9.69±0.20                 | 9.48±0.12                 | 9.59±0.15                 | 9.74±0.16                 | NS      | NS     |
| weight (g) 210 d     | 229.95±5.29 <sup>c</sup>  | 258.13±5.13 <sup>b</sup>  | 287.54±5.13 <sup>a</sup>  | 223.64±5.29 <sup>c</sup>  | <0.0001 | 0.0200 |
| SGR                  | 2.67±0.02 <sup>c</sup>    | 2.78±0.02 <sup>b</sup>    | 2.89±0.02 <sup>a</sup>    | 2.65±0.02 <sup>c</sup>    | <0.0001 | 0.0034 |
| Condition factor     | 1.44±0.02 <sup>a</sup>    | 1.46±0.02 <sup>a</sup>    | 1.48±0.02 <sup>a</sup>    | 1.38±0.02 <sup>b</sup>    | 0.0005  | NS     |
| Phase II (211-300 d) |                           |                           |                           |                           |         |        |
| Final weight (g)     | 661.66±14.92 <sup>b</sup> | 709.60±14.76 <sup>a</sup> | 730.23±10.48 <sup>a</sup> | 650.35±11.66 <sup>b</sup> | 0.0003  | 0.0001 |
| AGR                  | 4.07±0.14                 | 4.26±0.10                 | 4.17±0.10                 | 4.03±0.10                 | <0.0001 | 0.0001 |
| Condition factor     | 1.51±0.02 <sup>b</sup>    | 1.62±0.01 <sup>a</sup>    | 1.65±0.02 <sup>a</sup>    | 1.51±0.01 <sup>b</sup>    | <0.0001 | NS     |
| At harvest           |                           |                           |                           |                           |         |        |
| FCR                  | 1.71±0.09                 | 1.86±0.41                 | 1.73±0.17                 | 1.74±0.09                 | NS      |        |
| Survival             | 71.50±8.41 <sup>a</sup>   | 66.40±3.15 <sup>b</sup>   | 75.50±2.50 <sup>a</sup>   | 69.25±7.45 <sup>b</sup>   | 0.01    |        |
| Gross yield (kg)     | 55.45±5.17 <sup>a</sup>   | 47.10±4.90 <sup>b</sup>   | 61.72±4.90 <sup>a</sup>   | 52.85±5.23 <sup>a</sup>   | 0.03    |        |

## EFFECT OF GARLIC *Allium sativum* ON GROWTH AND IMMUNE SYSTEM OF *Clarias gariepinus*

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This study was carried out to investigate the effect of garlic on growth and immune system of *Clarias gariepinus*. A total of sixty juvenile fishes of approximately 51.00gram each was used. Fish samples were divided into four groups, A (control), B, C and D of fifteen fishes each, fed on garlic incorporated diet of 0%, 0.5%, 1% and 3% respectively. The control group was free from garlic. Fish in all groups was fed at a rate of 3% body weight twice daily for twelve weeks and it was readjusted as fish increased in weight. Fish growth in weight, total length and white blood cell (WBC) was measured weekly. Red blood cell (RBC), plasma protein, packed cell volume (PCV) and haemoglobin (Hb) were evaluated before and after the experiment. Results showed that there was no significant difference ( $p > 0.05$ ) in the final weight of fish samples in all concentrations. Blood parameters( white blood cell, red blood cell, packed cell volume and haemoglobin) of fish samples feed on all diet containing all levels of garlic inclusions were significantly higher than control while fish samples fed on diet containing 0.5% concentration of garlic was significantly higher( $p < 0.05$ ) than all concentrations. There was no significant difference ( $p > 0.05$ ) in the value of plasma protein of fish samples in all concentrations. It is concluded that garlic (*Allium sativum*) in diet of fish has no effect on weight but has effect on total length of fish and also increases white blood cell count there by improving the immune system of *Clarias gariepinus*.

## EFFECT OF FEED, FEEDING REGIMES AND STOCKING DENSITY ON GROWTH AND GONAD QUALITY OF *Tripneustes gratilla* UNDER FARM CONDITIONS

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Sea urchin gonads are used to prepare the Japanese delicacy called “Uni” and are one of the most expensive seafood items in the world. The total world production of sea urchin from capture fisheries reached its maximum in 1995 at 120,306 ton but had declined by 85% in 2005. This high rate of decline is as result of the high demand for their gonads which led to over-exploitation and depletion in their natural habitat prompting research on culturing sea urchin species.

*Tripneustes gratilla* will be used in this study as it is currently the most sort after species in Japan where it has high market acceptance. This study aims to determine the growth rates and marketable gonad quality of *T. gratilla* under farm conditions by using different feeds and feeding regimes. The macro-algae *Ulva amoricana* and *Gracillaria gracilis* will be fed along with a formulated diet. Sea urchins will be fed one of five feeds (fresh *Ulva* (FU), fresh *Gracilaria* (FG), a mixed feed of fresh *ulva* and fresh *Gracilaria* (FUG), and two formulated diets, one supplemented with 200 g dried *Ulva armoricana* (20U) and one without macro-algae (0U)) for 16 weeks to determine maximum somatic growth. A gonad enhancement trial will then be run for 8 weeks, where different combinations of the feeds will be tested on their effect to maximize the GSI and gonad quality (colour and texture).



## OIE'S APPROACH TO AQUACULTURE BIOSECURITY

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Aquaculture is growing globally more rapidly than all other food-animal producing sectors, and international trade in live aquaculture animals and their products is increasing. Development of the aquaculture sector, particularly in developing countries, is key to meeting the growing global demand for food.

Disease outbreaks due to unsafe trade or other factors such as poor farm-level biosecurity continue to cause serious economic impacts and threaten the growth and sustainability of the aquaculture sector.

Implementation of effective biosecurity measures is critical in reducing the risk of introduction, establishment and spread of pathogenic agents to, from or within an aquatic animal population.

The main focus of disease control should always be prevention. Implementing biosecurity measures in aquaculture establishments can be simple and have a significant impact to growth. Biosecurity measures include controlling what is brought into the farm, disinfection of aquaculture establishments and equipment, fallowing, ensuring quality of feed, and handling, disposal and treatment of aquatic animal waste.

The OIE *Aquatic Animal Health Code* provides standards for the improvement of aquatic animal health worldwide. Competent Authorities should use these standards to develop measures for early detection, internal reporting, notification and control of pathogenic agents in aquatic animals and preventing their spread via international trade in aquatic animals and aquatic animal products, while avoiding unjustified sanitary barriers to trade.

Biosecurity, a defined term in the *Aquatic Code*, is included in many of the *Code* chapters including the disease-specific chapters. The Aquatic Animal Health Standards Commission (AAC) is responsible for overseeing the amendments to the *Aquatic Code*. With the support of Member Countries it agreed to develop a new draft chapter on Aquatic animal biosecurity for aquaculture establishments under Section 4 of the *Aquatic Code*. The new chapter will contain general principles on aquatic animal biosecurity, address disease transmission pathways, risk assessment, mitigation measures and components of a biosecurity plan.

## **20-20 VISION, LESSONS FROM A FAILED OFFSHORE FISH PROJECT IN MOZAMBIQUE AND HOW THESE MAY BENEFIT THE EMERGING TILAPIA INDUSTRY IN EAST AFRICA**

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The coastline of East Africa provides a significant opportunity for commercial offshore fish production due to its massive scale, excellent site opportunities and stable tropical water temperatures. Despite this, there has been little commercial offshore aquaculture activity in the region to date and it would appear that this is mostly a result of the complexities of operating a large-scale investment in many of the countries concerned. Our view is that the high level issues are predominantly focussed on policy, licencing, financing, supply chains and distance from markets. Nonetheless, the Aquapemba fish farming project was initiated in 2008 as a pilot venture to produce Dusky Kob/Mulloy ( *Argyrosomus japonicus* ) in Pemba Bay, Mozambique. Early successes and promising results of juvenile production and growth rate/FCR led to further expansion up to a semi commercial sized project (200 tons per annum). This included partners from both South Africa and Scotland that were already involved in successful aquaculture projects. Unfortunately, the project was not able to capitalize on its results and many of the external factors highlighted above became the inhibitors for full funding into the commercial phase. As a result the project was shut down after 6 years of operations despite having a business plan backed by a track record and operational success.

Recently, the authors have become involved in assisting with the development of the Tilapia industry in the great lakes region of East Africa and have been able to apply some direct perspectives from the lessons learnt in Mozambique. This paper will discuss our views on what can be learnt from our past and how this can add to the resource pool. We believe that it is of prime importance that future failures of large projects are avoided so that the industry can develop and build confidence from external investment and funding organisations.

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## **INTEGRATION OF AQUACULTURE INTO AN INDUSTRIAL EFFLUENT TREATMENT SYSTEM AND THE RECOVERY AND REUSE OF WATER AND NUTRIENTS**

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This presentation will include a summary of the research program aimed at the recovery of water and nutrients from brewery effluent using sustainable water treatment technologies. The program covers the use of anaerobic digestion, algal ponding systems and constructed wetlands and the integration of crop irrigation, hydroponic vegetable production, aquaculture and alternative methods of separating algae from the treated effluent as part of the treatment process. The overall aim is to establish alternative sources of water for aquaculture in water scarce areas, while developing effluent treatment processes that result in down stream, income-generating products.

## **HOW TO DEVELOP MSC/PHD CURRICULA THAT ACCOMMODATE STUDENTS FROM A DIVERSE RANGE OF FOUNDATION TRAINING?**

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The preparation that university students receive prior to post-graduate studies varies between universities; yet, not all institutions acknowledge these differences and not all institutions accommodate these differences. This is a real problem in South Africa where resources and capacity have not always been equally distributed among institutions and this is particularly the case in specialised fields such as aquaculture and fisheries science.

In this presentation, I will use the post-graduate BSc Honours, MSc and PhD curricula in aquaculture and fisheries science at Rhodes University as an example. I will describe how our courses are structured and implemented, I will point out some strengths and weaknesses in our system, and I will generate a discussion that revolves around possible ways of addressing the concerns that we face in educating MSc and PhD students that have received vastly different foundation training.

## THE USE OF A MULTI-SPECIES PROBIOTIC DURING THE EARLY DEVELOPMENTAL STAGES OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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High temperature trout farming in South Africa pose severe stress and disease challenges that may impair production efficiency and viability. One aspect of concern is fingerling quality being stocked in grow out systems, as the condition of fish in their early stages of development ultimately influences performance during grow out. Restrictions in the use of prophylactic antibiotics limits control of opportunistic pathogens responsible for growth impairment and disease. The administration of probiotics during the early stages of development can potential is a solution to these problems.

A trial was conducted to evaluate the application of probiotics (*Lactobacillus casei*, *Lactobacillus plantarum*, *Rhodopseudomonas palustris*, *Saccharomyces cerevisiae*; Bactomune, Nutritionhub South Africa) during early stages of development of Rainbow trout (yolk-sac fry to 10g). The trial was performed in two phases:

- 1) a three-treatment post hatch phase (yolk-sac to Swim-up; 30 days) with water-addition of probiotics at a low (1.5 ml probiotic/liter water) and high level (3 ml probiotic/liter water) as well as a control treatment without water-added probiotics.
- 2) Six-treatment post swim-up phase (90 days) as a follow-up on phase one treatments with or without probiotic addition (5ml/kg feed) to the feed,

During the Yolk-sac stage the fish were sampled daily to examine yolk-sac efficiency and consumption rate. After swim-up stage the fish were sampled weekly to examine growth rate and FCR, and at the end of the trial the anterior intestine morphology was also evaluated in terms of section area, villi length, villi density, and goblet cells. Results will be presented.

## FEASIBILITY OF USING INDOOR-PAIRING CAGES FOR DEVELOPING COMPATIBILITY AMONG THE ORNAMENTAL MARINE DECAPOD, *Lysmata debelius*

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The enticing beauty of marine ornamental decapods makes them one of the most demanded species in the ornamental fish keeping industry. The increasing demand forces the suppliers to adopt unethical methods to collect the shrimps from their natural habitat. This leads to severe mortality of the animals and also has negative impact on the health of the reef ecosystem from where these are collected extensively. Developing captive breeding technology of the required species to ensure the sustainable supply is the only way out for the industry to sustain. In recent years, efforts have been made in many parts of the world to develop suitable protocols for the in lab production of marine invertebrates. As part of the Centre for Marine Living Resources and Ecology (Ministry of Earth Sciences, Government of India) funded project entitled “Establishment of a production cum training centre for ornamental fin and shell fishes at Fisheries Station, Puduveypu”, trials on the captive breeding of ornamental decapods was carried out. Present study deals with the indoor-cage techniques used for developing compatibility among the most coveted species, the cleaner shrimp, *Lysmata debelius*. It is commonly known as ‘fire shrimp’ which comes under Hippolytidae family. This species is an inhabitant of Indo-Pacific coral reefs. Captive breeding of fire shrimp is extremely difficult because of its peculiar sexual behaviour. It is a simultaneous hermaphrodite and highly aggressive to each other while kept together, hampering the smooth breeding process. The aim of the study was to develop a methodology for making compatible pairs of fire shrimp. Animals used for the trial were collected from the South East coast of Indian Ocean and brought to the hatchery at Fisheries Station, Puduveypu. The aquarium tanks used for rearing were rectangular in shape with 3x2x1 ft dimension holding 150 litres of water. The pairing cages designed for compatibility trials were made with netlon having the mesh size of 5x5 mm with five chambers each, measuring 16x12x22 cm length, breadth and height which were kept immersed in the aquarium water and stocked with individual shrimps. The photoperiod manipulation, specific feeding patterns and scientific time management in the interaction period between the individuals etc. facilitated the development of compatibility among the shrimps and subsequent mating. The details are discussed in the paper.

## ANAESTHESIA IN FISH AFTER PRE-DOSING WITH THE ANAESTHETIC AT A LOW CONCENTRATION FOR A SHORT PERIOD

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Anaesthesia in fish has been achieved with a wide range of chemical compounds with the aim of rapid induction of anaesthesia and safe recovery in the shortest possible time. Many experiments on the use of anaesthesia were designed to estimate induction time and recovery time as a function of the concentration of the compound used in order to establish a dosage response function.

We report on the use of a range of anaesthetics in several fish species. Fish were exposed to a low non-sedative concentration of the selected compound of approximately 10% of the tested dosage for 2–3 minutes. This was followed by exposure to a concentration that was known to safely anaesthetise the fish. Thus, experiments were designed to a) expose fish to a low concentration with the rest of the anaesthetic being added immediately after short exposure, or b) to expose fish to the full concentration for the same amount of time. Time to induction, time to recovery, survival and behavioural responses were recorded.

The data suggest that in some species recovery time can be shortened in pre-exposed fish when compared to fish receiving the full dosage in one administration. Thus, without increasing the amount of anaesthetic used, this simple change in procedure may lead to faster recovery from anaesthesia.

Based on the fact that both time of exposure *and* concentration influence recovery time, we suggest using the product of the concentration (e.g., mg L<sup>-1</sup>) of an anaesthetic and the duration of exposure (e.g., seconds) as a unit of reference in order to standardise values from different experimental conditions and designs.

The methods, and differences between fish species and anaesthetics will be discussed by comparing the results from five experiments in which this method was tested.



## THE USE OF CRYSTAL MENTHOL AS AN ANAESTHETIC IN TILAPIA *Oreochromis mossambicus* AT THREE WATER SALINITIES AND TWO TEMPERATURES

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Fish anaesthesia has been used for a wide range of applications, such as weighing and measuring of fish, fish transport, or surgery. We report on the first use of crystal menthol as an anaesthetic for juvenile tilapia *Oreochromis mossambicus* at three water salinity levels and two temperatures.

To test the effect of salinity on average induction and recovery times, fish were exposed individually to 85 mg L<sup>-1</sup> crystal menthol dissolved in ethanol at three salinity levels (0, 15 and 35 ppt) at 20 randomly assigned independent replicates per treatment. Fish in freshwater were induced significantly faster than those kept at the other two salinities, while average recovery times did not differ between treatments. In the freshwater treatment, the average times (seconds) for induction and recovery were 227 ± 47 s, and 326 ± 124 s, respectively. At the highest salinity, average time to induction and recovery were 343 ± 75 s and 371 ± 171 s, respectively. These values were similar to those recorded at the medium salinity, i.e., 359 ± 60 s and 367 ± 160 s, respectively.

The effect of temperature on induction and recovery times was tested by exposing 30 fish individually to 85 mg L<sup>-1</sup> of crystal menthol at 21 °C and 30°C. At 30°C, fish were anaesthetised within 340 ± 21 s, which was 110 seconds faster than at 21 °C (450 ± 39 s). The fish at the higher temperature recovered significantly faster (251 ± 15 s) than those at 21°C (316 ± 12 s). In both experiments, there were no mortalities.

Regression models to predict recovery rate as a function of duration of exposure will be also be presented.

Crystal menthol may be used as an anaesthetic in this species as it achieved fast anaesthetic induction times, fast recovery and no mortality.

## TEACHING EXPERIMENTAL DESIGN, DATA ANALYSIS AND SCIENTIFIC WRITING AS PART OF THE THIRD-YEAR ICHTHYOLOGY CURRICULUM AT RHODES UNIVERSITY, SOUTH AFRICA

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Generally, undergraduate students are “end-users” of scientific knowledge. They open a textbook to read and obtain “knowledge”, but they have rarely been part of the process that generated information. Students may also use knowledge from different sources and, if the information is sufficiently controversial, take a standpoint on which view to support, i.e., produce an essay. I report on my experience of teaching third-year students the tools to “obtain knowledge” as a result of a scientific investigation and, importantly, to make a switch from just exercising an assigned task, i.e., a research project, to owning this knowledge, due the fact that the students are asked to design the experiment. In my experience, this process can be taught at this level and yield publication-quality outcomes.

This eight-week course consists of four sections. In the first two weeks, I present the basic concepts of experimental design and methods of data analysis. This is followed by a period during which the students conduct pilot experiments under guidance. Using this experience, we discuss how to improve the experimental design and the methods. Each group of 3–4 students presents a research proposal to its peers. This is usually followed by intense discussions, fostering independent thinking and the exercise of fair criticism. The students conduct the main experiment over 2–3 weeks. We then analyse and interpret the data together in small groups and prepare a scientific manuscript.

In my experience, when the data set that needs to be analysed is *owned* by the student as a result of an experiment, which they have conceptualised, there is a very strong interest in data analysis. I suggest that the success of teaching experimental design, data analysis and scientific writing depends on the level of ownership of the information obtained during research.

## MOLECULAR CLONING AND EXPRESSION PATTERNS OF TUMOR NECROSIS FACTOR ALPHA GENES IN CRUCIAN CARP *Carassius carassius* FOLLOWING BACTERIAL INFECTION

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TNF $\alpha$  is known as a pleiotropic and potent proinflammatory cytokine, which is produced by several cell types including macrophages, monocytes, leukocytes, mast cells, and smooth muscle cells in response to inflammation, infection, and other physiological challenges. TNF $\alpha$  upregulates the production of other inflammatory cytokines and eliminate bacterial and viral pathogens by phagocytosis and chemotaxis. Disease problems have grown proportionally with the intensive culture of aquaculture species. Bacterial disease motile aeromonad septicemia caused by *Aeromonas hydrophila* is responsible for serious morbidity and mortality in marine and freshwater aquaculture.

Fish were acclimatized to laboratory conditions for at least 7 days prior to bacterial infection.

12 fish were intraperitoneally injected with 0.1 ml of the *Aeromonas hydrophila* BSK10 strain ( $5 \times 10^7$  cells/mL). 3 fish from each tank were randomly sampled at 6, 12, and 24 h post the bacterial injection. Total RNA was extracted from tissues tested before and after infection.

Two types of the TNF  $\alpha$  gene (TNF $\alpha$ 1 and TNF $\alpha$ 2) were cloned from crucian carp. Real time PCR analysis showed a constitutive expression of crucian carp TNF $\alpha$ 1 and TNF $\alpha$ 2 in all seven tissues examined (Figure 1). The TNF $\alpha$ 1 was expressed significantly higher in the liver and kidney than those of TNF $\alpha$ 2, while TNF $\alpha$ 2 in turn was expressed significantly higher in the muscle.

The *Aeromonas hydrophila* upregulated the expression level of both TNF $\alpha$ 1 and TNF $\alpha$ 2 in all of the tested tissues. At 6 h the expression levels of TNF $\alpha$ 1 were increased significantly higher in the muscles, skin, and liver, (Figure 2) while the expression levels of TNF $\alpha$  2 were increased significantly higher in the muscles and gills. TNF $\alpha$ 1 was expressed much stronger than TNF $\alpha$ 2. At 12 h the expression levels started to decline and were even lower at 24 h. These results imply that both TNF $\alpha$ 1 and TNF $\alpha$ 2 mRNA are distributed differently in tissues and are implicated in the immune response to bacterial infection.

(Continued on next page)

FIGURE1.Expression patterns of *TNF*α-1 and *TNF*α-2 genes in tissues before bacterial infection

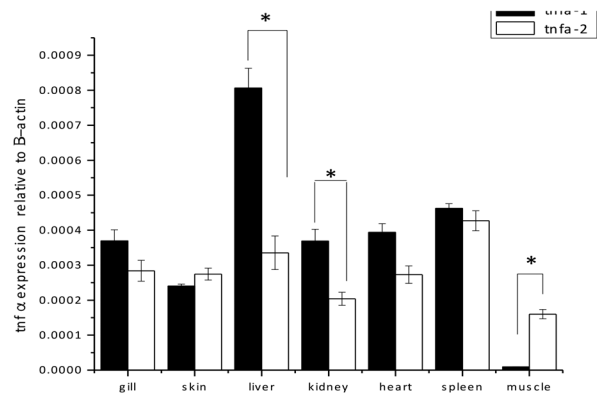
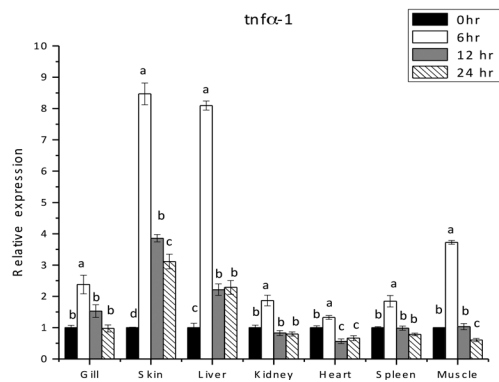


FIGURE 2. Expression levels of *TNF*α-1 in tissues at 6, 12, and 24 h after bacterial infection with *Aeromonas hydrophila*.



## **TOWARDS A HARMONISED PHD REGIONAL CURRICULUM IN AQUACULTURE AND FISHERIES SCIENCES: LESSONS FROM LUANAR CURRICULUM REVIEW PROCESS**

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The world has changed significantly since the Millennium Development Goals (MDGs) were embraced in 2000. A comprehensive approach to fisheries and aquaculture is also well depicted in Sustainable Development Goal 14 (SDG 14). Several targets of SDG 14 call for specific actions in aquaculture and fisheries that include: effectively regulate harvesting; end overfishing and Illegal Unreported and Unregulated (IUU) fishing; address fisheries subsidies; increase economic benefits from sustainable management of fisheries and aquaculture; provide access for small-scale fishers to resources and markets. Hence, Higher Education curriculum is called upon to respond accordingly to these expectations. A new knowledge of sustainable development is not only required but should also be sufficiently responsive to the complexity and multiplicity of trajectories characterizing the Aquaculture and Fisheries Sector in the region. In view of this, the current PhD regional curriculum in aquaculture and fisheries sciences at LUANAR was reviewed to respond to the needs, complexity and changes in the region.

The curriculum review process included review of existing curriculum and policy documents, stakeholders' consultations, development of course content and validation of the updated curriculum. The findings showed differences and gaps in the course content, capacity to deliver the programs, mode of delivery and assessment procedures in the existing postgraduate curriculum in aquaculture and fisheries science in the region. It was also noted that there is no clear transition in course content from Master degree in aquaculture and fisheries sciences to PhD curriculum by course work for the same programme. It is therefore recommended that the curriculum for the regional programs should be harmonised in course content and assessment procedures among others. It is also important to share from the pull of resources in aquaculture and fisheries in the region to fill the gaps in the quality and delivery of the regional curriculum.

## **AREVIEW OF POLY CULTURE SYSTEMS AND THE IMPORTANCE OF POND POLY CULTURE WITH SMALL INDIGENOUS FISH SPECIES AS A PRO-POOR TECHNOLOGY FOR SMALL HOLDER FARMERS IN AFRICA**

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Aquaculture in Africa is generally characterized by the monoculture of selective species for commercial markets (tilapia, catfish, carp) with a few examples where these commercial species are grown in polyculture systems. Whilst polyculture has been practiced for centuries, especially in Asia, the benefits and potential to widen the scope of polyculture systems in Africa has not been recognized. This is particularly the case when assessing the potential of small indigenous species (SIS), which have been determined to contain superior micronutrient profiles compared to common commercial species such as tilapia. SIS are thus defined by their size but also by consumption patterns since they are generally consumed whole and have a range of health benefits. In Africa, aquaculture has struggled to meet its potential, compounded by the difficulty in accessing and growing single commercial species in extensive monoculture systems. This is especially true for poor, smallholder farmers. We provide a systematic review of inland polyculture systems from around the world with a specific focus on SIS and with the goal of providing theoretical and empirical data that shows the potential of pond polyculture to increase productivity and enhance food security. Most articles on polyculture are based in Asia and detail systems mixed with tilapia, catfish, and/or carp species with a few cases in Bangladesh that focus on SIS. There are a few cases of wetland species in fish ranching studies in the great lakes region of Africa. The review suggests that polyculture systems can be beneficial for farmers to increase productivity and use more sustainable methods of farm management. The review further suggests that, whilst little is known about the cultivability of many SIS, the potential to develop research around pond polyculture with SIS and the importance for productivity, consumption and nutrition are immense, as seen in many studies in Bangladesh. Finally, polyculture enables management techniques such as phytoplankton-based growth and partial harvesting, which are more complementary to the conditions and characteristics of African smallholder aquaculture. A higher diversity of fish, the use of indigenous species, and multi-trophic approaches to pond systems constitute a sustainable technology option that can be more compatible with African smallholder aquaculture. This may especially be the case for poor farmers that struggle to grow for commercial markets and where malnutrition and food security are still major development challenges. The potential to widen the parameters for species selection is important for the growth and development of aquaculture in the region. The successful development of polyculture systems however, depends on species selection and experimental studies. For this reason we also provide a useful species selection method conducted by WorldFish that screens for potential SIS based on availability, market value, nutrient value, role in dietary diversity and the potential for cultivability. It is hoped that the systematic review and screening method will enable more research into SIS polyculture systems and integrated, multi-trophic approaches to inland aquaculture in Africa.

## TRENDS IN AQUACULTURE VALUE CHAIN DEVELOPMENT IN SUB-SAHARAN AFRICA: THE CASE OF ZAMBIA

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Sub-Saharan Africa produces less than 1% of the world's fish supply from aquaculture. Despite this, aquaculture is still growing at 12.6% a year on the sub-continent and places like Nigeria, Ghana, Kenya, Uganda and Zambia are showing real growth potential. This paper presents details on the rapid rise of commercial aquaculture in Zambia, where the sector grew at an average of 41.7% per year (2008-2014). Farmed tilapia (99.2% of the market share) from intensive land-based pond systems and cage culture operators now produce over 71% of 20,000 mt of farmed fish, the rest coming from smallholder farmers. This has sparked new value chain trends, such as feed mills venturing into fish feeds and hatcheries producing indigenous (*O. andersonii*) and exotic species (*O. niloticus*) for culture. Farmed tilapia is now cheaper than all other animal source foods and only marginally more expensive than wild-caught tilapia from Zambian fisheries. Farmed tilapia in Zambia is generally consumed by a rising middle class in urban areas and sold to commercial retailers and supermarkets. New marketing channels are however becoming visible, such as the mostly female traders from the capital, Lusaka, who distribute lower grade, smaller-sized farmed tilapia to low-income areas in the city, albeit on a low scale at present. Smallholder farmers have meanwhile experienced stagnation in production in the last decade due to familiar problems seen in much of the rest of the sub-continent, namely, a lack of access to high quality inputs, services and markets. This is further compounded by the fact that most rural farmers are banned from utilising the fast-growing exotic *O. niloticus* species outside production zones that are located in the south of the country, where the bulk of the commercial sector is located. The ban is criticized to benefit the commercial sector at the expense of rural farmers. Zambia's rising aquaculture industry has played a role in increasing the fish per capita consumption rate to 11kg per capita in 2014 (Figure 1). However, a dramatic increase in imported farmed fish from Asia has played a more significant role in raising the per capita consumption rate and without fish imports, this rate would go down by 3.9kg in 2014, well below the 8.9kg average for Africa. It remains to be seen what affect the import of farmed tilapia products from Asia will have on the competitiveness of the aquaculture industry in Zambia. Other countries in the region are likely to experience similar market-led expansion trends in aquaculture in the next years.

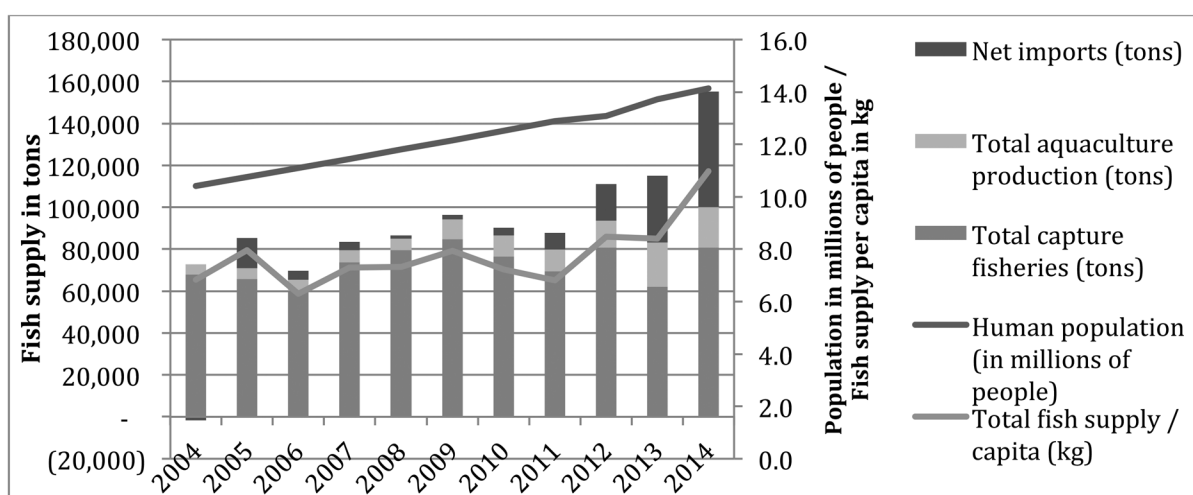


FIGURE 1: Total fish supply and supply per capita (2004-2014)



## AMAZON TILAPIA *Oreochromis niloticus* LEATHER DYED WITH AÇAÍ *Euterpe oleracea* EXTRACT

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Tanneries have been reported as a great cause of environmental impact, due to the pollution generated by their residues. Chemical dyes, when left untreated, can damage fauna, flora and even human populations beyond visual pollution and ecosystem changes. Açaí extracts possess dye properties that, besides resulting in an intense color, are a natural and abundant residue of native vegetal extraction in the Amazon region, which also has a diversity of barks, seeds, fruits and leaves that can be used as natural dyes. This research aimed to evaluate the dye obtained from açaí extracts in dyeing Nile Tilapia leather as a more economical and environmentally correct alternative source for leather tanning activities in the northern region of Brazil.

The present study was carried out at the Texturas da Amazônia tannery, in Rondônia, in September 2016. A total of 250 Tilapia skins ranging from  $700 \pm 1.2$  grams of body height from fish farms certified for cultivation in net tanks were evaluated. The skins were tanned in vegetable tannin and dyed at a ratio of 15% of açaí extract in relation to skin weight. The açaí pressing residues used for human consumption were dried in the shade, followed by immersion in water and ethanol for 48 hours to extract the dye from the açaí bark. The dyed leathers, which were stabilized in 18% of moisture obtained by drying at room temperature for 4 days, were evaluated with regard to the Light Solidity test, migration in pvc coatings and washability. All tests were performed by the MK Química do Brazil Laboratory following ABNT standards. The parameters were evaluated using a scale from 1 (very bad) to 5 (very good). Washability was achieved by washing the skins 15 times alternating with soaking for 30 minutes and 15 minutes under running water. The tilapia skins dyed with the açaí extract presented stability with regard to visual characteristics, and were classified as very good, scale 5, concerning migration in pvc and regular, scale 3, regarding light solidity. Concerning leather aspects after washing in water, good fixation and color definition, scale 4, (Figure 1) were observed, demonstrating that açaí extracts meet the norms of natural dyes in terms of good fixation in relation to light, perspiration and washing, and thus, are considered efficient with regard to the concentration and extraction mode of the residue.

Açaí extracts, thus, hives the potential to be used as an alternative dye source for dyeing tilapia leather.

Figure 1. Tilapia leather dyed with açaí extract depicted during the drying process.



## RESISTANCE OF PIRARUCU *Arapaima gigas* SKIN TANNED IN DIFFERENT PLANT TANNIN CONCENTRATIONS

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*Arapaima gigas*, known as Giant of the Amazon, since it reaches up to 300 kg in body weight, is the second most cultivated species in Rondônia, Brazil. This state produces 9 thousand tons/year of this fish slaughtered at the average commercial weight of 12 kg, with approximately 14% of scaly leather. The use of plant tannins as a replacement for chromium in pirarucu leather tanning seeks to aggregate the use of regional products in tanning costs and reduce environmental impacts. The aim of this study was to evaluate the physical-mechanical resistance of Pirarucu skin tanned at different plant tannin concentrations.

The study was carried out at the Texturas da Amazônia tannery industry, Rondônia, in January 2017. Twenty pirarucu fish were used, presenting  $14 \pm 1.2$  kg body weight from a certified excavated nursery. The hides were tanned at 5%, 10%, 15% and 20% plant tannin concentrations, and, after reaching 18% humidity, lengthwise and crosswise bodies of evidence from the dorsal region of the fish were removed and submitted to physical-mechanical analyses according to ABNT standards, by the MK chemistry of Brazil Laboratory. The mean of the Pirarucu dermis thickness ranged from 2.0 to 4.7 mm. The increase in tannin concentrations reduced tensile strength and increased the progressive tearing of Pirarucu leather, although they did not influence elasticity (resistance to elongation until rupture). At 15% tannin the tanning did not influence lengthwise leather tensile strength ( $P < 0.05$ ), of 15.99 N/mm<sup>2</sup>, while the other concentrations presented lower crosswise resistance (table 1). The tear rate was significantly higher, of 10.8 N/mm, in the crosswise direction of the leather at 5% tannin. At 15% tannin greater leather physical-chemical stability for the preparation of garments and artifacts from the leather of Pirarucu was observed.

Table 1. Resistance to traction, elongation and tearing of *Arapaima Gigas* leather tanned at different levels of plant tannin.

| Tannin concentration | Leather direction | Tensile to traction (N/mm <sup>2</sup> ) | Resistance to Stretching (%) <sup>3</sup> | Tear strength (N/mm) |
|----------------------|-------------------|--|---|----------------------|
| 5%                   | Lengthwise        | 20.67 a                                  | 86.50                                     | 8.68 b               |
|                      | Crosswise         | 13.18 b                                  | 93.75                                     | 10.85 a              |
| 10%                  | Lengthwise        | 12.88 a                                  | 83.50                                     | 12.02                |
|                      | Crosswise         | 4.78 b                                   | 101.0                                     | 11.93                |
| 15%                  | Lengthwise        | 15.86                                    | 91.00                                     | 12.01                |
|                      | Crosswise         | 16.12                                    | 79.25                                     | 12.59                |
| 20%                  | Lengthwise        | 12.18 a                                  | 82.50                                     | 13.79                |
|                      | Crosswise         | 16.12 b                                  | 84.50                                     | 12.74                |
| VC <sup>1</sup> (%)  |                   | 4.24                                     | 3.56                                      | 2.20                 |
| P-value              |                   | 0.0399                                   | 0.0975                                    | <0.0001              |

<sup>1</sup>CV: Variation Coefficient. Letters in the columns differ 0.05% by the Tukey test. Regression equations:  $^2\hat{Y} = 16.18750 - 0.98500 \cdot T$  ( $R^2 = 97.5$ ),  $^3\hat{Y} = 85.78$ ,  $^4\hat{Y} = 7.56250 + 0.056650 \cdot T$  ( $R^2 = 98.0$ )

## THE IDENTITY OF *Ulva* (ULVACEAE, ULVOPHYCEAE) SPECIES IN COMMERCIAL AQUACULTURE IN THE SOUTH AFRICAN ABALONE INDUSTRY

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As of recent times *Ulva* L. has been one of the most studied genera, firstly because of wide applications in aquaculture as well as the taxonomic problems that saw the incorporation of the species formerly ascribed to *Enteromorpha* Link. *Ulva* species can be very difficult to identify due to the few and often variable characters. This has resulted in wrongly documented distribution records with the accompanied difficulty of verifying this. The thalli found in the abalone farms are unattached and thus even more difficult to identify due to the lack of polarity. In this study we used molecular data, nuclear ribosomal internal transcribed spacer DNA (ITS nrDNA) ITS and the chloroplast encoded *rbcL*, to establish which species of *Ulva* are cultivated in the abalone industry in South Africa. Based on a total of 69 ITS sequences and 65 *rbcL* sequences inclusive of those (21 and 26, respectively) from Genbank, we found that at least four different species are cultivated in the South African abalone farms and these represented species *U. stenophyloides*, *U. armoricana*, *U. rigida* and *U. capensis*. The study also found that the specimens that have locally been ascribed to *U. lactuca* and widely believed to be the main crop in the local abalone farms did not correspond to *U. lactuca* as recorded elsewhere. The only records of “true” *U. lactuca* were collected from the Cape Town harbour, pointing to a likely recent introduction.

## REPRODUCTIVE PERFORMANCE AND SEX RATIO OF NILE TILAPIA *Oreochromis niloticus* FED DIETARY *Aspilia mossambicensis* AND *Azadirachta indica* LEAF MEAL

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Prolific breeding is a major setback for farming mixed sex Nile tilapia *Oreochromis niloticus*. Various techniques including the use of synthetic hormones have been proposed to overcome this challenge. However, most of the techniques are highly hampered by cost, skills requirement and health concerns which makes their application difficult especially to small scale fish farmers. Therefore, this calls for a need to search for natural agents as a safe and affordable alternative. The present study was conducted to investigate the effect of two medicinal plants, Wild sunflower *Aspilia mossambicensis* and Neem, *Azadirachta indica* on the reproductive performance and sex ratio of *Oreochromis niloticus*.

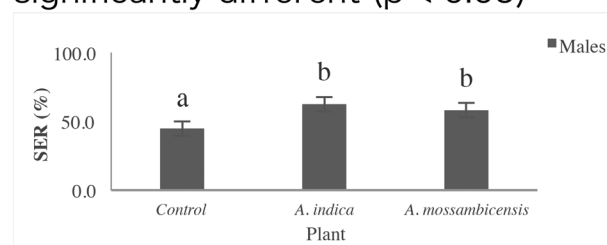
*O. niloticus* juveniles ( $41.5 \pm 3.1$ g mean weight) were used for reproductive performance experiment. Experimental diets AM1, AM2, AM4, AM8 and AI1, AI2, AI4, AI8 were formulated by adding 1.0, 2.0, 4.0 and 8.0 grams of *A. mossambicensis* and *A. indica* leaf meal respectively to a kilogram of control diet (25% CP). Fish were then fed at a ratio of 3% body weight for 90 days. Reproductive performance was determined in terms of hatchlings count (HC), absolute fecundity (AF), relative fecundity (RF), gonadosomatic index (GSI) and histology of the gonads. On the other hand, two weeks old hatchlings were used for sex reversal experiment. Experimental diets were made by adding 40 grams of *A. mossambicensis* and *A. indica* leaf meal to a kilogram of control diet. Fish were fed with experimental diets for 90 days, followed by control diet for the remaining 60 days at 20% of their body weight. Feeding was done two times a day (10.00 and 17.00 hours).

Results revealed significant ( $p < 0.05$ ) decrease in HC, AF and RF of fish fed *A. mossambicensis* and *A. indica* leaf meal doses (Table 1). Accordingly, histological alteration of gonads was noticed in both males and female fed doses 4.0 and 8.0 g kg<sup>-1</sup>. On the other hand, the two plants significantly ( $p < 0.05$ ) altered the 50:50 (males:females) sex ratio (SER) in favor of males (Figure 1). Findings indicate that *A. indica* and *A. mossambicensis* can control prolific breeding as well as reverse the sex of *O. niloticus*.

TABLE 1: Reproductive performance of *O. niloticus* fed *A. mossambicensis* and *A. indica* doses. Values in the same column sharing the different superscript are significantly different ( $p < 0.05$ ).

|         | HC    | AF    |      | Males | Females |
|---------|-------|-------|------|-------|---------|
| Control | 259.4 | 698.3 | 10.7 | 2.5 ± | 3.4 ±   |
| AM1     | 149.4 | 361.0 | 3.8  | 1.6 ± | 4.5 ±   |
| AI1     | 100.3 | 459.0 | 7.4  | 0.9 ± | 4.3 ±   |
| AM2     | 121.2 | 444.3 | 4.7  | 1.6 ± | 3.7 ±   |
| AI2     |       | 346.7 | 5.6  | 1.1 ± | 3.9 ±   |
| AM4     | 94.2  | 372.3 | 4.8  | 2.3 ± | 5.8 ±   |
| AI4     | 76.1  | 343.3 | 6.6  | 1.1 ± | 3.8 ±   |
| AM8     | 101.9 | 383.3 | 4.5  | 2.1 ± | 4.7 ±   |
| AI8     |       | 364.0 | 4.7  | 1.3 ± | 4.5 ±   |

FIGURE 1. Sex ratio for males *O. niloticus* fed *A. mossambicensis* and *A. indica* doses. Values sharing the different superscript are significantly different ( $p < 0.05$ ).



## **TWENTY YEARS OF AQUACULTURE IN NORTH AFRICA: DEVELOPMENTS, CRITICAL ASSESSMENT AND FUTURE**

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This paper presents the evolution of aquaculture in North Africa during the 20 last years, and the assets and bottlenecks for each country. This work uses the results of the European project Aquamed (2011–2013). The analysis of the evolutions of this sector, including research and development allows to establish a diagnosis on the strengths and the weaknesses in the four countries (Morocco, Algeria, Tunisia and Egypt). The retrospective analysis helps in identifying solutions for a more sustainable development. It also contributes to select priorities for action in a long-term vision (2035). This double overview, on the past and the future, should help decision-makers and experts to identify the best politics for a sector, which has a high potential in the region. Research capabilities and issues and development prospects are assessed using a prospective approach.

The analysis of 20 years of aquaculture research and development in North Africa allows two major lessons: the development of the sector is always linked to the conjunction of precise and known favorable conditions (market, environment, livestock systems, species) and a critical mass of reactive decision makers. The clearest example is that of Egypt, where the emergence of favorable conditions has made it possible to increase tenfold aquaculture production in 20 years. On the other hand, when a reception framework is prepared with a long-term vision involving the main actors, the projects benefit from favorable context, which reduces the risk of blockage, facilitates procedures and accelerates implementation.

For this, the Mediterranean aquaculture multistakeholder platform, set up within the framework of the Aquamed, and «mirror» national and regional platforms are particularly useful. These «mirror» structures are constituted as Aquamed through a network involving all stakeholders in aquaculture: producers, institutional and researchers. The dynamics created is then to maintain by adapting research to problems who appear. In this context, international cooperation and training are necessary and must be sustained.

In a networked world connected to multiple databases, the added value of any project is the quality of work on the integration of knowledge into a dialogue between all actors, the capitalization of experience, the anticipation of crises, and finally the ability to strategic choices by the prospective approach. North African countries now have access, and collectively, to this potential. It depends on them to value it, building on the experience gained and the willingness collectively build sustainable aquaculture.

## **DETERMINATION AND MITIGATION OF RISKS OF PATHOGEN/DISEASE INTRODUCTION INTO AQUATIC ANIMAL FACILITIES: CASE SCENARIO**

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Biosecurity can be defined as a system of processes (i.e., inputs, movements and other activities), each with a set of procedures, that taken together minimize the risk of introduction and spread of infectious organisms within or between aquatic animal populations. Biosecurity measures at the site level include bioexclusion (prevention of pathogens from entry), within-site infectious disease control (management of pathogens within a facility) and biocontainment (prevention of pathogens from release). This presentation will focus on bioexclusion of pathogens/diseases from commercial aquaculture facilities. Viral hemorrhagic septicaemia virus (VHSV) in Atlantic salmon will be used as an example.

A risk assessment is used to estimate the risks of introducing pathogenic organisms or disease into an aquatic animal site and provide recommendations for mitigation of the risks. Sources of disease introduction include aquatic animals, water, fomites, vectors and feed. An on-site analysis outlines the scenario for potential pathogen introduction into the site. This includes working knowledge of the production biology of the species, the physical layout of the facility and the process flow for operations. The on-site analysis also identifies mitigation measures in place to reduce the risk to negligible, as well as gaps where mitigation measures should be in place.

A documented biosecurity plan is the only way of demonstrating that effective disease control is in place without having to implement an ongoing effective testing plan. After the risk assessment is complete, the site can produce a biosecurity plan outlining the identified points of pathogen introduction and the mitigation measures to reduce the risk of introduction to negligible. Standard operating procedures (SOP's) are developed for each of the mitigation measures using a standardized format. The plan includes all records necessary to document the appropriate implementation of the mitigation measures. Plans are typically reviewed once yearly.

## DNA EXTRACTION PROTOCOLS MAY INFLUENCE BIODIVERSITY DETECTED IN THE INTESTINAL MICROBIOME: A CASE STUDY FROM WILD PRUSSIAN CARP, *Carassius gibelio*

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In this investigation, we examined the influence of different DNA extraction protocols on results obtained for intestinal microbiota of Prussian carp (*Carassius gibelio*). In our work, we use two common commercial kits to extract total DNA from seven fish intestines (mucosa and chyme separately): AxyPrep Multisource Genomic DNA Miniprep Kit (Union City, CA, USA) and KitDNA sorb B (Moscow, Russia).

It was shown that the microbiota of Prussian carp using the Axygen Kit was dominated by Proteobacteria and Firmicutes while using the DNA-sorb B kit the dominants were Proteobacteria, Firmicutes and Bacteroidetes. The NMDS using Bray-Curtis similarities between the samples showed a clear grouping of the samples from intestinal mucosa and content extracted by DNA-sorb B and of the same samples extracted by AxyPrep.

Moreover, ANOSIM is supported by these results and indicated that the composition of OTUs was significantly different among all the communities, based on each factor: type of tissue and type of extraction (Table). For type of tissue, R value was 0.266 ( $P = 0.001$ ), for type of extraction R value was 0.477 ( $P = 0.001$ ).

Table. Two-way ANOSIM pairwise comparisons of bacterial community composition using Bray–Curtis values. Bootstrapping with 1000 resamplings.

| Group  | Global R | Significance |
|--|----------|--------------|
| Type of tissue (intestinal mucosa vs intestinal content) | 0,266    | 0,001        |
| Type of extraction (DNA sorb B vs Axygen)                | 0,477    | 0,001        |

For supporting our results we ran the meta-analysis (correspondence and non-specific correlation Gamma test at  $p \leq 0.05$  analyses) of 25 published articles describing the biodiversity of freshwater fish. We found that intestinal microbiota of fish depend on many different factors, including the type of DNA extraction which is supported by our experimental results.

Microbial diversity, community structure, proportions of read numbers derived from each OTU and the total number of OTU's obtained by different DNA extraction protocols could lead to a bias in results obtained. Therefore, we recommend two significant points in bacterial community studies of fish gut: 1) whenever possible divide samples for microbiota analyses into their constituent parts, mucosa and chyme (intestinal content) to enable identification of autochthonous bacteria resident in the gut from those merely passing through; and 2) combine several extraction methods, if possible, to recover some of the observable biodiversity that is lost when using only one method. Consequently, knowing these possible sources of bias exist, researchers should be conservative in conclusions about structures of bacterial community of wild and cultured populations of fish species.

This work was supported by the Russian Foundation for Basic Research, project no.16-34-00309.



## FISH-PREY RELATIONSHIPS IN A MODEL SHALLOW STEPPE LAKE: FROM BACTERIAL COMMUNITY POINT OF VIEW

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The diversity of the intestinal microbiota of fish is influenced by environmental factors such as feeding habits, temperature, salinity, and others (Ringo et al., 2016). However, the correlation between gut microbiota and the hosts environmental microbiota is not fully understood. The aim of this study was to estimate possible correlations between gut microbiota of eight fish species and prey microbiota (chironomid larva (Chironomidae), daphnia (Daphniidae), water boatman (Corixidae), backswimmer (Notonectidae), amphipod (Gammaridae), caddis fly larva (Trichoptera), spiny water flea (*Bythotrephes longimanus*), diving beetle (Dytiscidae), water mite (Hydrachnidae)), and environmental microbiota (water, sediment and reed periphyton) in nature by using 16S rDNA sequencing (Illumina, MiSeq). Material was collected in June–July of 2012 in Malye Chany Lake and estuaries of its tributaries. Malye Chany Lake is a shallow, eutrophic lake in Western Siberia (Russia, 54°37' N, 78°09' E).

Analysis of diet in eight fish species in Chany Lake based on Morista-Horn index are shown following feeding groups: 1) omnivorous: Prussian carp *Carassius gibelio*, Crucian carp *Carassius carassius* and common carp *Cyprinus carpio*; 2) zoobenthivorous and zooplanktivorous: roach *Rutilus rutilus*, dace *Leuciscus leuciscus* and ide *Leuciscus idus*; 3) piscivorous: perch *Perca fluviatilis*, pike-perch *Sander lucioperca*.

The microbiota of mucus and content of fish stomach (perch and pike-perch) and intestine (all species), prey and environment were dominated by Proteobacteria, Bacteroidetes, Actinobacteria, Fusobacteria and Cyanobacteria (Fig 1). The diversity analysis of microbial community has shown the decline of Chao index as follow: «environment» > «preys and intestinal content» > «stomach and intestine mucosa and stomach content». Principle component analysis showed a clear grouping of the samples into 4 groups: 1) stomach and intestinal mucosa, 2) stomach and intestinal content, 3) preys and 4) environment (Fig. 2). Fish gut microbial community is strongly divided on bacteria that associated with content and mucus for all studied fish regardless the gut organization (gastric/agastric) and feeding habits.

For aquaculture purposes we recommend to divide samples for microbiota analyses into their constituent parts, mucosa and chyme whenever it is possible.

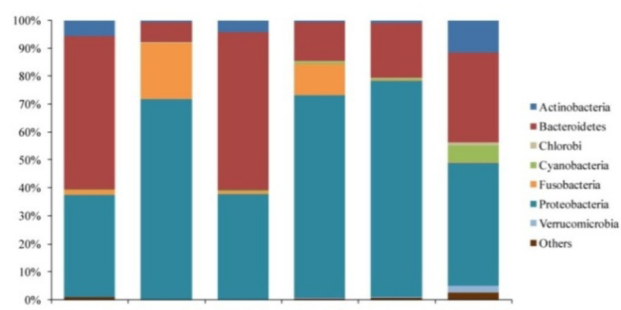


Figure 1. Phylum composition of stomach and intestine microbiota of fish with different feeding habits and prey and environmental microbiota.

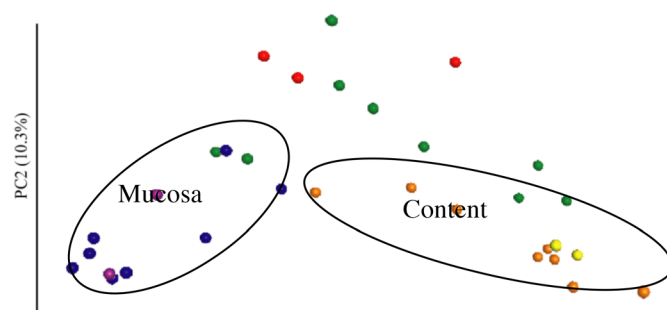


Fig. 2. Principle component analysis. Blue – intestinal mucosa, orange – intestinal content, purple – stomach mucosa, yellow – stomach content, green – prey and red – environment.

## **AFRICA CENTRE OF EXCELLENCE IN AQUACULTURE AND FISHERIES (AQUAFISH): EXPLORING INNOVATIVE WAYS OF TEACHING AND CURRICULUM DEVELOPMENT IN ITS POSTGRADUATE PROGRAMMES**

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The Aquaculture and Fisheries Science Centre of Excellence (AquaFish), based at Lilongwe University of Agriculture and Natural Resources (LUANAR), seeks to train a pool of skilled and innovative graduate students to contribute towards improved fish-based food and incomes from aquaculture and fisheries in Africa. The establishment of AquaFish is in line with, and will significantly contribute to the Comprehensive Africa Agriculture Development Programme (CAADP), the Science, Technology and Innovation Strategy for Africa (STISA), and the Malawi Growth and Development Strategy, to increase human capacity development for aquaculture and fisheries sector growth. These development frameworks recognize the need to increase fish production, to improve fish per capita consumption and subsequently reduce malnutrition and stunting that are prevalent in the region. AquaFish centre builds on LUANAR's track record of serving as a regional training centre in aquaculture and fisheries science for Africa. LUANAR hosts the regional PhD programmes in Aquaculture and Fisheries Science, and Agricultural and Resource Economics on behalf of 60 member universities of the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM). In addition LUANAR (the Aquaculture and Fisheries Science Department) is a designated Regional Fish Node responsible for advancing science and biodiversity research within the SADC region under the NEPAD Southern Africa Network on Biosciences (SANBio). AquaFish centre will facilitate and promote scaling-up graduate training in Aquaculture and Fisheries, community action research, and strengthen linkages with the private sector in the region while increasing females and youth participation. The centre will employ innovative, entrepreneurial and multidisciplinary approaches to training, research and outreach on production, value addition and fisheries management, through strategic south-south and north-south partnerships with advanced knowledge institutions and other higher education stakeholders. The main outcomes of the centre will include: (i) enhanced capacity to produce graduates that are relevant to industrial needs in aquaculture and fisheries sector in the region, (ii) fit-for-purpose proactive graduates (338 MSc and 84 PhD) to support aquaculture, natural resource management and nutrition, and (iii) strengthened partnerships with industry, research and academia in the production of quality graduate students in the aquaculture and fisheries value chain, relevant for economic development.

## **TO PARTICIPATE IN INFORMAL FISH TRADE OR NOT; THE CASE OF BUSIA (UGANDA/ KENYA) BORDER**

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The study investigated factors influencing the choice of fish traders' marketing channel and determinants of fish trader's gross margin. A multistage sampling technique 115 fish trader, 4 key informants and 2 focus groups who participated in the study. A Probit model, Gross margin analysis, and Ordinary Least Square (OLS) regression were used to determine factors that influence the choice of fish traders' marketing channel, compare profitability of the marketing channels and determine factors that influenced traders gross margin respectively. Findings from the study revealed that volumes of fish traded per month, distance to market, membership to a fish marketing organization, payment mode, household size, presence of other sources of income were statistically significant in determining traders' choice of marketing channel. In addition, it was found that formal traders on average sold 6.882 tons of fish whereas 2.095 tons of fish were sold by the informal traders per month. Despite the fact that the formal traders sold large volumes of fish compared to informal traders, the study indicated that informal traders made 4 percent more profit than formal traders. Further analysis showed that Age of fish trade, Household size, quantity of labour used, access to credit, purchasing and selling prices affected fish trader's gross margins significantly. The study therefore recommends that traders lobby for funds to buy cooling trucks to reduce on the cost of transportation, plus preservation costs that greatly lower formal trader's gross margin, harmonise policies concerning fish trade in the East African community that are put in place. In addition, the study recommends that policies for example, frequent transfers for the Beach Management Unit (BMU) officials be put in place so as to reduce corruption among them. Investment in infrastructures such as roads and establishment of more ice plants will increase traders' gross margin.

## **TO PARTICIPATE IN INFORMAL FISH TRADE OR NOT; THE CASE OF BUSIA (UGANDA/ KENYA) BORDER**

Mastulah Kawala, Theodora Shuwu Hyuha Ekere William, Peter Walekwa, Gabriel Elepu,  
Sloans Chimatiro Kalumba

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## RECENT STATUS AND ADVANCES ON AQUACULTURE AND GENETIC BREEDING ON IVORY SHELL *Babylonia areolata* IN CHINA

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The ivory shell, *Babylonia areolata*, is dioecious mollusk that is mainly distributed across tropical and subtropical coastal areas in Southeast and East Asia. It is also considered as a recently emerging maricultured species. In recent ten years, the aquaculture area is expanded gradually, while the aquaculture area singly in Hainan Province had reached 600,000 square meters in 2014 according to the statistics, with annual economic benefit of nearly 350 million RMB. However, because of intensive breeding and the breeding environment was worsened, leading to the high mortality, thus causing great economic losses. In 2011, our group launched the inheritance and selection research of *B. areolata*.

This research analyzed and compared the protogenesis traits of two geographic populations of *B. areolata* on the basis of growth and survival data of 12 months' age and taking *B. lutosus* as control group, and carried out mass selection, pedigree selection and crossbreeding on the basis of it. This study analyzes the selective response and realized heritability of three successive generations of *B. areolata* through mass selection; it analyzes and compares the difference of important quantitative traits between Hainan population and Thailand population, and applies microsatellite to evaluate the genetic variation level of these two populations after successive selection; it evaluates the genetic parameters of important economic traits of *B. areolata* with pedigree selection method; it researches the hybridization effect of *B. areolata* by taking two populations as materials and with diallel crossing method, and evaluates the interaction effect between four genotypic groups and the environment at the same time.

The new strains which had better growth trait and stronger stress resistance have obtained through the breeding project. These research would be better to solve the problems occurred during breeding process at present, and make *B. areolata* industry achieve better development.

IMPLICATIONS OF SALMON FARMING IN DISPERSIVE COASTAL SITES – ARE THESE ENVIRONMENTS BETTER SUITED TO COPE WITH ORGANIC WASTES?

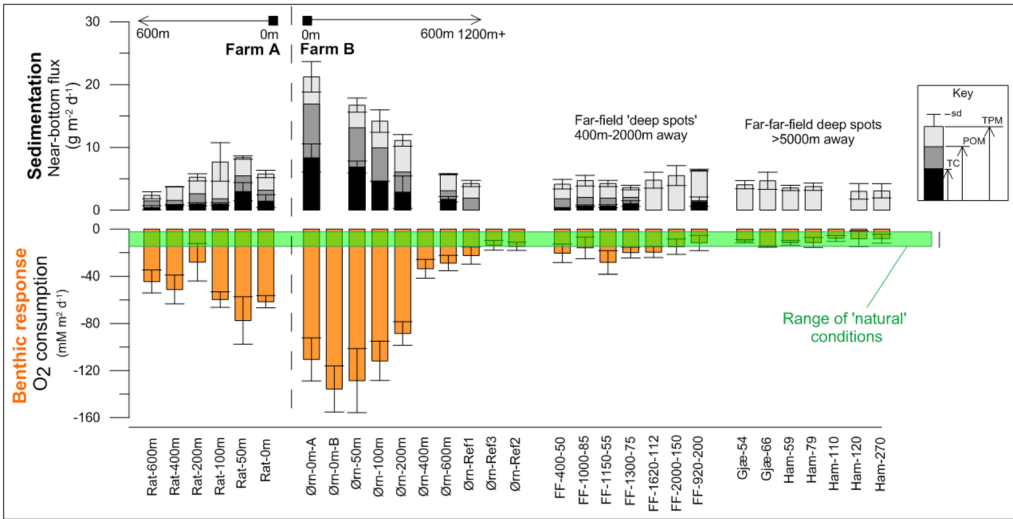
Nigel Keeley, Raymond Bannister, Skye Woodcock, Thomas Valdemarsen, Marianne Holmer

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Finfish farming in cages is associated with high organic sedimentation and significant ecological impacts in the seabed beneath, and can result in adverse changes in sediment chemistry, biodiversity and associated benthic indicators. There is a global trend to relocate fish farms from sheltered inshore areas, to more physically dynamic and dispersive coastal ecosystems, because of assumed increased resilience to organic enrichment. However, the exact processes by which the effects are mitigated, and the associated changes in ecosystem functioning, are poorly understood.

This talk outlines new results from a comprehensive field study linking organic matter sedimentation to changes in benthic biogeochemistry and fauna diversity along a spatial gradient away from a salmon farm situated in a well-flushed, semi-exposed coastal site in Norway. The farm has supported very high finfish production for >20 years, and visual observations of the coarse sandy seabed have only ever indicated minor organic enrichment. However, our detailed measurements revealed a strong benthic enrichment gradient, evident in both the infauna community and sediment metabolic processes. Benthic oxygen consumption and nutrient release were strongly stimulated within 200m of the pens, where the infauna was extremely prolific; yet changes in some traditional enrichment indicators (%OM & redox) were relatively unresponsive. Changes in benthic metabolism were clearly linked to farm-derived sedimentation, although only a portion of the farm waste was accounted for by local benthic processes. Benthic functioning was clearly altered 600m away, with possible effects also observed in far-field ‘deep spots’.

This study provides new insight into ecosystem functioning and resilience in dispersive coastal ecosystems. Benthic footprints of salmon farms tend to be less severe, very productive and larger in spatial extent, and therefore have greater assimilative capacity. This information is essential for better positioning and environmental management of fish farms in the future and provides some much needed validation points for the use of predictive depositional models in dispersive environments.



**Table 1:** Coupled near-bottom sedimentation and benthic oxygen flux rates at multiple near-field, far-field and far-far-field sampling stations around two fish farms, Frøya, Norway.

## LARVAL-REARING TECHNIQUES FOR DUSKY KOB *Argyrosomus japonicus* WITH A SPECIFIC FOCUS ON A NOVEL FEEDING REGIME

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One of the biggest limiting factors in marine finfish aquaculture is the low survival rate of early-stage larvae. Most mortalities can be ascribed to the poor nutritional value of live feeds, sibling cannibalism, and various stressors that result in swim bladder hyperinflation and/or starvation during the larval stage. Research results vary on the best timing for the introduction of artificial feed for good survival and growth rate in dusky kob larvae. The main objective of this experiment was to improve survival and growth rate. The experiment focused on a new feeding regime that sought to wean larvae onto an artificial diet earlier than the current *Argyrosomus japonicus* standard (weaning commenced at 16 days after hatch versus 20 days after hatch), based on findings and recommendations made by Musson & Kaiser (2014). An earlier weaning period should increase survival rates, while reducing the labour costs associated with a lengthy period of live feed cultivation. A major concern relating to the earlier weaning time that was reported by other authors was that the reduced amount of *Artemia* that the new feeding regime would receive would have a negative effect on larval growth rate (Ballagh *et al*, 2010).

Three trials were conducted, each with five replicate tanks subjected to the new feeding regime and five replicate tanks subjected to the standard feeding regime used in the local marine finfish industry in RSA in a fully randomised design. Water quality variables were measured daily. Samples of fish from each tank were collected every two days for the duration of the trial. Morphometric measurements (standard length; body depth; eye diameter) obtained from these larvae were used to compare growth rate between treatments. Tank survival rates were calculated on the last day of each trial.

Morphometric data obtained in all three trials during this experiment showed no significant difference in growth rate between treatments. Survival rate did not differ significantly between treatments in any of the three trials.

### References:

- Musson M, Kaiser H (2014) Development of the digestive system in dusky kob, *Argyrosomus japonicus*, larvae. *Aquacult Int* 22: 783-796
- Ballagh D A *et al.* (2010) Weaning requirements of larval mullet, *Argyrosomus japonicus*. *Aquaculture Research* 41, 493-504



THE IMPACT OF MOBILE TECHNOLOGY BASED EXTENSION SERVICES: THE CASE OF FISH FARMERS IN GHANA

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Aquaculture in sub-Saharan Africa is practiced by a majority of rural farmers who lack access to reliable information. The role of information in the success of aquaculture is indispensable. In Ghana, per capita fish consumption is over 25kg annually. However, there are challenges to meeting this demand through aquaculture. This has been attributed to a number of factors including inadequate extension delivery, irregular formal and informal farmer training resulting in poor feeding practices, improper water quality management, etc. These coupled with the high extension officer to farmer ratio makes farm visits challenging. However, mobile phone platforms such as text and voice messaging could serve as a means to improve fish farmers access to information. Therefore, this pilot study was conducted to assess the impact of voice messaging in reducing the knowledge gap of fish farmers.

The Human Centered Design Approach was employed in a baseline study in three selected regions of Ghana after which a questionnaire was administered to 90 pond fish farmers to ascertain their information needs. Subsequently, information on best management practices (BMPs) in fish farming were developed in consultation with an extension officer before recording in a local dialect for voice messaging. This information was sent to 500 fish farmers located in the selected regions on weekly basis for 24 weeks through their mobile phones.

The baseline survey revealed that, out of the 90 farmers surveyed: 77% prioritized access to aquaculture best management practices while 23% prioritized access to market prices and input supplies.

At the end of the pilot, 50% of the fish farmers engaged showed interest to pay for the mobile-based extension service. The fish farmers rated the service as follows; 33.3% of the farmers rated as excellent, while 44.4% as very good and the remaining 22.2% rated the service as good. The Impact Assessment results revealed that the total fish production increased by 44% (Figure 1) as a result of the mobile-based extension services.

In conclusion, through mobile based extension services fish farmers will be able to access information on best management practices, at any time of the day, in their preferred local language.

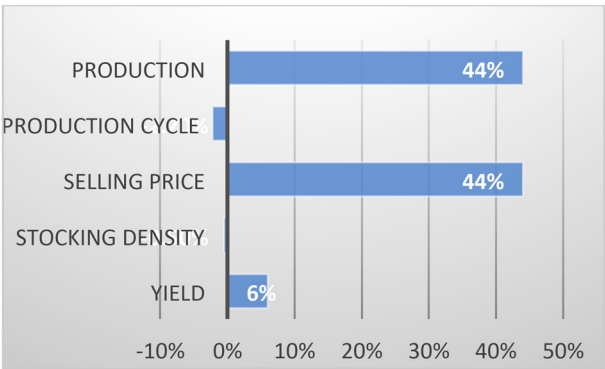


Figure 1. Percentage change Impact Assessment Indices after the pilot.

## AVAILABILITY AND PRICE VARIABILITY OF OILSEED MEALS USE IN AQUAFEED IN GHANA

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High prices and limited supply of fishmeal, the conventional protein source in most animal feeds have led to the search for alternative protein sources in feeds. Oilseed meals and by-products have been suggested as potential fishmeal replacers with regards to their high nutritional value. However, information about their availability and price variability is scanty especially in Sub-Saharan Africa. This study assessed the availability and price variations of groundnut meal, palm kernel cake, soybean meal, groundnut testa (skin), copra meal and cottonseed meal in Ghana, through semi-structured questionnaires.

At the end of the survey, sources of the oilseed meals were found to be dominant in the agro-ecological zones where the respective oilseed crops are cultivated (Figure 1). The results showed that all the selected oilseed meals were available in large (>1000 MT) quantities across the country except cottonseed meal.

Estimated production quantities of the oilseed meals ranged between approximately 9,000 MT and 66,000 MT with soybean meal being the highest produced, although, the second most expensive after groundnut meal. Prices of all the selected oilseed meals generally, have a persistently rising trend varying from GHS 0.20 to GHS 6.60 per kg. Factors that were found to account for the variability were fuel price and production quantities of the oilseeds (Table 1).

In conclusion, the oilseed meals excluding cottonseed meal are readily available in substantial quantities but the relatively high price of groundnut meal makes it uneconomic for aquafeed usage in Ghana in terms of cost and sustainability.

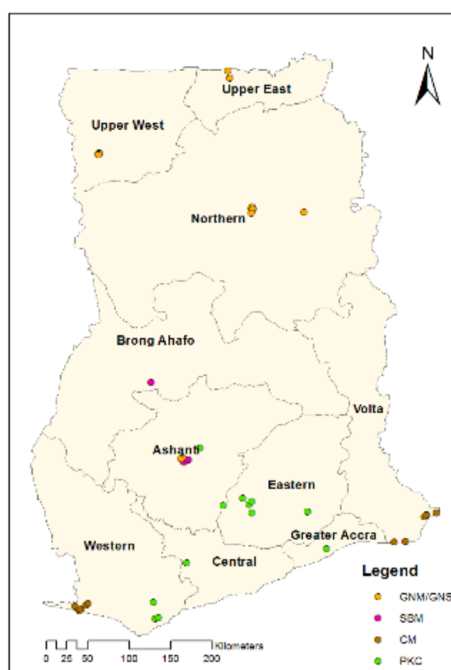


FIGURE 1. Sources of Groundnut meal (GNM), Soybean meal (SBM), Copra meal (CM), Palm kernel cake (PKC) and Groundnut skin/ testa (GNS) in Ghana.

## **NATIONAL STRATEGIC ENVIRONMENTAL ASSESSMENT FOR AQUACULTURE DEVELOPMENT IN SOUTH AFRICA – A SYNOPSIS OF THE CURRENT MARINE AND FRESHWATER AQUACULTURE ENVIRONMENT AND THE NEED TO PROMOTE SUSTAINABLE GROWTH AND INCENTIVISATION**

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Globally aquaculture is becoming increasingly important due to the global demand for fishery products, and as the supply from capture fisheries have been decreasing in recent years it is anticipated that the shortfall in demand will come from aquaculture. In South Africa, the aquaculture industry is still in its developmental stage in comparison to the global aquaculture community, however, it has the potential to grow and contribute towards job creation, food security, economic development and export opportunities.

To date, several factors have contributed to the suboptimal development of the marine and freshwater aquaculture sector in South Africa. Not only is this industry considered inherently overregulated but it also faces various environmental, economic, social and technical challenges. Some key challenges include the requirement for numerous regulatory authorisations, the production demand being focused on a few high-value species, scarcity of freshwater and a harsh marine environment, difficulty in accessing project funding, limited pool of skills and support services, unpredictability associated with climate change, extreme variance in seasonal temperatures, challenges with access to sufficient land and sea space, and perceived competition with the tourism and conservation sectors.

During 2016, a national-scale Strategic Environmental Assessment (SEA) was commissioned by the South African government addressing these challenges to create a suitable enabling environment where aquaculture development can be promoted and incentivised. The SEA will aim to achieve its purpose in two ways; (i) to identify optimal aquaculture development areas using multi-layered spatial analysis of the receiving environment, and (ii) to provide the competent authorities with a streamlined and integrated management and regulatory framework to reduce compliance complexities and improve decision-making processes pertaining to these identified optimal aquaculture areas. The SEA is being conducted over 18 months, concluding in December 2017, and includes both freshwater and marine aquaculture.

In this paper, the approach to the SEA is presented, with an emphasis on how the SEA will assess and scale the various aquaculture environments, the associated production systems employed, the priority species selected and the regulatory framework governing this sector.

## TILAPIA FARMING IN SOUTH AFRICA – PROBLEMS AND SOLUTIONS

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Tilapia farming in South Africa has only been made commercially viable since the inclusion of *oreochromis niloticus* as a species for aquaculture having previously been banned as alien and invasive. The average South African climate constrains the species to enclosed greenhouse intensive RAS production techniques with the three best provinces for growing the species currently refusing to issue permits. Government bureaucracy, red tape and strangulation of the industry continue to undermine it's stated objectives via Operation Phakisa and within this context a fledgling industry is struggling.

Solutions are explored in this presentation including getting government officials skilled, up to date with RAS technology and the deployment of large scale investment in the sector.

I further cover solution based approaches by small farmers utilising intensive scaleable farming systems that can scale into larger production operations over time.

Development of aquaculture skills is also of paramount importance to the industry and these will be in short supply as solutions to the government induced headaches manifest themselves.

I finish with an overview of large scale technologically driven aquaculture production systems and how they despite the initial capital costs are able to generate profitable sustainable tilapia production simultaneously co-existing with small scale farmers as a possible model for African aquaculture in the future.

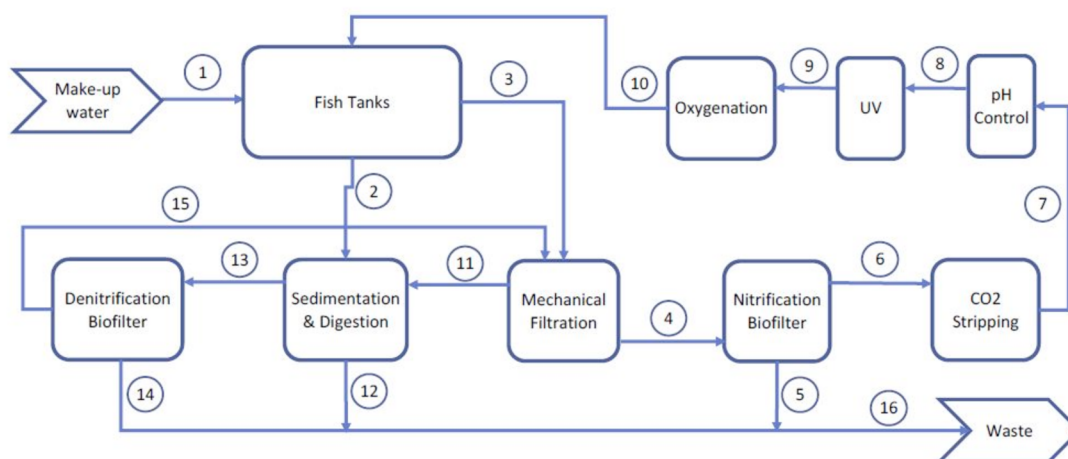


Figure 1: Basic Process Flow Diagram for Tilapia RAS in Africa

## COMPARATIVE STUDIES OF BETA GLUCAN AND PLANT STIMULANTS ON THE GROWTH AND IMMUNE RESPONSE OF *Labeo rohita*

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The experiment was conducted to evaluate the effect of beta glucan and plant stimulants on the growth, body composition and hematological parameters of *Labeo rohita* (Rohu), reared for 120 days in flow through system. Fish with an average body weight of 250g were stocked in cemented tanks @ 10 fish/replicate. Four iso-nitrogenous artificial feeds containing 0.1% of beta glucan, plant stimulant, plant+bacteria and control (without any immunostimulants) were given @ 3% body weight to *Labeo rohita* twice a day. Significant ( $P<0.05$ ) difference in weight gain was observed between the control and plant stimulant treated group while it was non-significant ( $P>0.05$ ) for the other treatments. The total serum proteins and cell count showed a non-significant ( $P>0.05$ ) difference among all the treatments. The body composition analysis revealed that plant stimulant treatment group has significantly higher content of crude protein and lower fat compared to control. In conclusion, the plant stimulants can be used without any adverse effect on total serum proteins, hematology and fish body composition.

**Table 1. Growth studies of *Labeo rohita* under various treatments of herbal and bacterial immunostimulants.**

| Parameter<br>s  | T1                           | T2                            | T3                           | T4                            |
|-----------------|------------------------------|-------------------------------|------------------------------|-------------------------------|
| Ini.wt (g)      | 252.55±0.4<br>5 <sup>b</sup> | 260.35±1.0<br>5 <sup>ab</sup> | 270.85±8<br>.3 <sup>a</sup>  | 274.95±0.7<br>5 <sup>a</sup>  |
| Fi.wt (g)       | 341.25±0.8<br>b              | 349.62±0.4<br>8 <sup>ab</sup> | 355.92±6<br>.78 <sup>a</sup> | 353.82±0.0<br>2 <sup>ab</sup> |
| Wt. gain<br>(g) | 88.7±1.3 <sup>a</sup>        | 89.27±0.57<br>a               | 85.07±1.<br>57 <sup>a</sup>  | 78.87±0.77<br>b               |
| FCR             | 4.7±0.1 <sup>c</sup>         | 4.95±0.05b<br>c               | 5.15±0.1<br>5 <sup>b</sup>   | 5.60±0.10 <sup>a</sup>        |
| SGR (%)         | 0.3±0.0 <sup>a</sup>         | 0.3±0.0 <sup>a</sup>          | 0.3±0.0 <sup>a</sup>         | 0.2±0.0 <sup>b</sup>          |

## NOVEL NON-ESTROGENIC ENDPOINTS OF PHENOLIC METABOLITES TOXICITY IN FISH: USING ZEBRAFISH AS A MODEL FOR STUDY

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Evidence of the severe effects of phenolic metabolites on different fish species exists in a number of axes including endocrine and non-endocrine as well. Regardless the classical issues of such metabolites as endocrine disrupting chemicals, novel non-estrogenic points are poorly studied. This article demonstrates the non-estrogenic attribution of certain phenolic metabolites in fish species. Confirming this non-estrogenic action of such compounds, zebrafish (*Danio rerio*) embryos were subjected to different doses of Polybrominated diphenyl ethers (BDEs) to study the effect of such class of chemicals on the fish embryogenesis during the first 120 hours of fertilization.

## **POVERTY ERADICATION THROUGH FRESH WATER AQUACULTURE AND INLAND FISHERIES DEVELOPMENT IN BOTSWANA**

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The Government sees the development of Fisheries and Aquaculture sectors in Botswana as one of the key priorities with others. The Government also foresees the role of fisheries and aquaculture to enhance food security, eradicate poverty, generate employment, improve rural livelihoods and increase investment. The aquaculture project initiatives by the small-scale fish farmers and the business sector as a whole will further enhance/complement a number of on-going activities launched by the Office of the President in collaboration with other relevant line Ministries. The objectives of this project is a) to increase income and nutritional status of rural communities as add-value through increased production of farmed fresh water fish in line within NDP 10 & 11 and VISION 2036 (Income/Nutrition); b) to empower targeted enthusiastic communities by providing fish feed and fingerlings through the establishment of integrated freshwater small-scale fish farms including aquaponics/hydroponics, cage farming etc. in the selected districts of Botswana (Commercial Viability). It would focus on: HIV/AIDS & lactating women, orphan vulnerable children, people living with disabilities, fish farmers, youth, cooperatives, small medium entrepreneur SME etc.

Fish farming and Fresh Water Aquaculture in Botswana is infant stage and characterized mainly by fish extraction from the country's main water bodies with minimal contribution to poverty eradication. An Aquaculture Advisor has been engaged to join the Poverty Eradication Coordinating Unit at Office of the President to provide advice to the development of fish farming projects. The Advisor has started off with a pilot project in order to paying field visits to potential fisheries and aquaculture sites in the country to be established fish farming projects consisting of construction of fish ponds, renovation of existing hatchery, establishment of mini fish feed plant, introduction of integrated farming (fish and ducks), build-up aquaponics/hydroponics farming at households levels, setting up of a horticultural garden and an orchard within the same premises using reclaimed waste water. The project will be operated by or to the benefit of poverty eradication beneficiaries and will be replicated to other parts of the country where sufficient waste or borehole water can be found to sustain the operation.

Towards the end of 2015, Lake Ngami Conservation Trust was assisted with funds amounting to P4 140 000 to exploit the fish resource by setting up fish processing plant, cold storage, 2-3 fish landing stations/fishing camps, fish outlets, procure refrigerated truck. The beneficiaries will also be trained to commercialise fish farming. These will entail the provision of fish ponds, fish cages, procurement of the required fishing equipment and its accessories such as gill nets, freezers, etc. Fish will be introduced and reared in man-made ponds and like in the main water bodies at various places in the country, it will be caught using gill nets, will be processed, packaged and stored in deep freezers for marketing. The project will also involve training of beneficiaries in Fisheries and marketing of the products. Beneficiaries will be assisted to access the local market for fish, such as local lodges and local communities.

A budget amounting to P54 Million has been requested for a series of tasks for the plan period (3 years' timeframe from 2016-2019 FY) i.e. deployment of consultants and technicians, renovation of existing hatchery, site assessment exercises (Soil. Water, EIA etc.), feasibility study/business plan, establishment of 6 small-scale fish farms, 6 integrated fish farms, 1 commercial fish farm, set-up aquaponics/hydroponics model at households level, build-up mini hatchery and mini fish feed plant, procurement of fish farming logistics (plastic pond liner, water quality test kit, borehole digging, green house materials, solar power, oxygen aerators etc.), capacity building (fisheries and aquaculture training need assessment study, review of existing training materials and development of field-tested extension materials in English as well as other local vernacular languages, in-country training of trainers on aquaculture techniques for the trainers, simple training on aquaculture techniques, business plan and record keepings for the farmers, people living with disabilities, youth, cooperatives and SME group etc. regional and international aquaculture study tours and tailor made aquaculture training course for the high officials and best performing farmers, international conference and the seminars for the officials to exchange and share their project outcomes.



## CULTURE OF POLYCHAETES AS AN ALTERNATIVE SOURCE OF MARINE FISHMEAL AND FISHING BAIT

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While aquaculture is the fastest growing animal production system, tropical mariculture and capture fisheries, continue to perform below expectation, with consequences on ecosystems and livelihoods. Mariculture quality feeds require high protein and fatty acid content, and unregulated harvesting of fishing baits, such as polychaetes, with consequences on ecosystem and consequently on performance. Temperate polychaete culture protocols, as alternative fishfeed and baits, are available, however local solutions are lacking.

This study attempts to artificially culture and test a tropical eunicid polychaete (*Marphysa mossambica*), commonly exploited by artisanal fishers as bait. Preliminary results of polychaete performance under a variety of feeding (spinach, manure) and growing regimes (intensive, semi-intensive) were monitored and presented here, prior to subsequent evaluation as fishfeed and bait.

Preliminary results are shown in the table and suggest that highest survival (<90%) and growth (<0.7 g.d<sup>-1</sup>) is achieved under manure diets and semi-intensive regimes. Although there were moderate rates of survival under intensive regimes, recruitment was only observed in semi intensive regimes.

It is concluded that although intensive systems have been adopted elsewhere, tropical eunicid perform best under manure diets in semi-intensive regimes. Further elaboration of the eunicid biology is needed prior to developing, adapting and refining, local appropriate culture techniques.

| <b>Regime</b>         | <b>Survival<br/>(%)</b> | <b>Growth<br/>(g.d<sup>-1</sup>)</b> |
|-----------------------|-------------------------|--------------------------------------|
| <b>Spinach</b>        | 60.5                    | 0.43                                 |
| <b>Manure</b>         | 96.7                    | 0.66                                 |
| <b>Mixture</b>        | 61.4                    | 0.43                                 |
| <b>Intensive</b>      | 62.5                    | 0.37                                 |
| <b>Semi-intensive</b> | 93.3                    | 0.68                                 |

# INVESTIGATION OF GENE EXPRESSION PATTERN OF ZONA PELLUCIDA DOMAIN 4 (ZP4) IN PACIFIC ABALONE *Haliotis discus hannai* AND ITS EXPRESSION IN EARLY STAGE OF THE GONAD DIFFERENTIATION

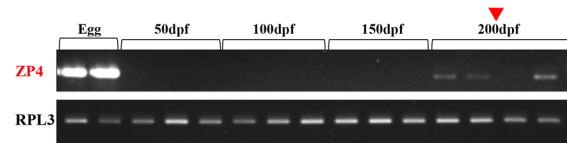
Jong-Myoung Kim\*, Mi-Jin Choi, Kyung Myo Kang, Han Kyu Lim

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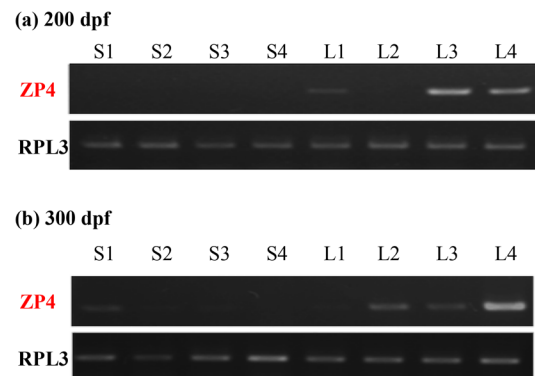
Several genes were shown to be exclusively expressed in female or male gonads have been reported in teleosts. To study the mechanism of sex differentiation in marine invertebrate, especially in economically valuable marine invertebrate, pacific abalone *Haliotis discus hannai*, molecular markers for determining the sex determination are need to be developed. From transcriptome analysis of *H. discus hannai*, zona pellucida domain 4 (ZP4) was shown to be expressed in a female gonad and to be related with a growth rate of abalone. In order to understand further the sexual maturation of abalone and its relationship to the growth, RT-PCR was carried out with RNAs isolated from egg, and gonads collected from abalones of 50, 100, 150, and 200 days post fertilization (dpf). ZP4 transcripts were shown to be detected from gonads of female abalones from 200 dpf. The frequencies of abalone expressing ZP4 transcript in their gonad will be analyzed with different size abalones of various dpfs.

To examine the developmental stage-dependent expression of ZP4 together with sex differentiation, RNAs were isolated from eggs as a positive control and individual abalones collected at 50, 100, 150, and 200 dpf. Amplification of cDNA was carried out by using ZP4 specific primers and ribosomal protein L3 (RPL3) primer as an internal control. Developmental stage-dependent expression of ZP4 in abalone was shown by the detection of ZP4 in abalones from 200 dpf (Figure 1). The result indicates that the sex differentiation process to female abalone seems to start from 200 dpf.

Differential expressions of ZP4 were examined in different (small and large) size abalones collected from 200 (Figure 2-a) and 300 (Figure 2-b) dpfs. The result indicating a higher frequency detection of ZP4 in a large size abalone as compared to that of small size abalone suggest a faster growth rate of female abalone. This, together with its female specific expression, indicated that ZP4 may be used as a marker for identification of fast growth and female-specific sex determination in *H. discus hannai*.



**Figure 1.** Developmental stage-dependent expression of ZP4. RT-PCR was carried out with RNA isolated from soft tissues of abalones collected at the indicated dpfs.



**Figure 2.** Expression of ZP4 was detected from small (S1-S4) and large (L1-L4) sizes of abalones collected at 200 dpf (a) and 300 dpf (b).

## A STUDY ON THE POLYCULTURE OF SEA SQUIRT *Halocynthia roretzi* AND SEA CUCUMBER *Apostichopus japonicus* UNDER A HANGING CULTURE SYSTEM

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The southern part of the Korean sea is an important aquaculture ground because of its mild water temperature and many islands around that protect typhoons and strong waving waves. However, the changes in the natural environment and high-density cultures in a limited area have hampered productivity while caused mass mortality of species. This calls on us to develop eco-friendly farming technology so as to achieve sustainable aquaculture farms. To that end, it is necessary to develop aquaculture technology which not only helps the vertical use of sea surface and bottom but also improves environment in the sea cucumber poly culture. It is also necessary to develop poly-culture technology for oysters and sea squirt, the two major filtration-feeding culture species in the South coast and sea cucumber, a high value-added species functioning as a cleaner.

The average size of young sea cucumber released in April was  $3.7 \pm 2.9$  g. It continued to grow during the summer season and grew to an average of  $9.1 \pm 3.0$  g in December. The average size of adult sea cucumber released in April was  $31.5 \pm 5.6$  g in April. It became smaller to  $20.8 \pm 5.9$  g in early summer but grew back to  $28.8 \pm 13.8$  g in December. It has been confirmed that heavier sea cucumbers are more affected by high water temperature, so they sleep in the summer. Most of the sea cucumber were found to be under a shelter. From our night time survey on September 10, 2016, they were found out of the shelter. The density of sea cucumber had decreased to 4.2 individuals/m<sup>2</sup> until July since they were released from the shelter of the farms. Since then, it slowly decreased to be 3 individuals / m<sup>2</sup> in December. The survival rate of each shelter is 28.0% for A, 27.2% for B, 19.7% for C type,

DEVELOPMENT OF SPECIES-SPECIFIC PCR METHOD FOR THE IDENTIFICATION OF *Epinephelus septemfasciatus*, *E. bruneus* AND *E. akaara*

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This study was aimed to develop a rapid, precise and species-specific method of the identification of three grouper species (Red-spotted grouper, *Epinephelus akaara*; Longtooth grouper, *E. bruneus*; Seven-band grouper, *E. septemfasciatus*), by using polymerase chain reaction (PCR) mediated molecular method. A common universal forward primer and three different species-specific primers were designed for all the three species from the fragment of mitochondrial *cytochrome c oxidase I (COI)* gene. The species-specific amplification patterns showed three different bands in length of PCR products had 344 bp (Red-spotted grouper), 262 bp (Longtooth grouper), and 130 bp (Seven-band grouper) which were available in the DNA samples isolated from each verified species, unknown collections, and even in mixed processed samples. Thus PCR assay provides reliable, simple, easy and low cost method for species identification of these three species of groupers.

The species-specific amplicons obtained from the PCR reactions showed three different bands of basepair (bp) length, 344 bp for Red-spotted grouper, 262 bp for Longtooth grouper and 130 bp Seven-band grouper (Figure. 1). In addition to this, the results from the triplex PCR using the all four primers developed in this study yielded three different bands of PCR products from the single reaction. These results suggest that this method may be applicable for identifying the species of three fishes at molecular level.

The results from the verified specimens showed identical results as the preliminary identification of the species. In addition, the PCR analysis of the tail-cuts collected from wild specimens caught by local fishermen showed same information with those of phenotypic identification (Table 1). Further we found 13 Red-spotted groupers, 27 Longtooth groupers and 40 Seven-band groupers from the specimens collected from retail markets and commercial restaurants. Among those, three specimens of the Seven-band groupers were different from the species information provided by three different restaurants.

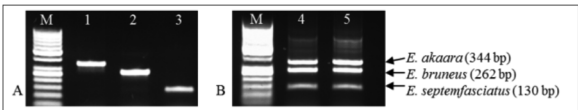


Figure 1. The species-specific amplicons obtained from the PCR reactions. PCR products showed three different bands of basepair length, 344 bp for Red-spotted grouper, 262 bp for Longtooth grouper and 130 bp for Seven-band grouper. B, PCR products from a single PCR reaction using a universal primer and three species-specific primers together for mixed fish foods.

Table 1. Identification of groupers using species-specific PCR analysis

| DNA samples        | No. of samples | Red-spotted grouper | Longtooth grouper | Seven-band grouper |
|--------------------|----------------|---------------------|-------------------|--------------------|
| Specimens verified | 215            | 172                 | 25                | 18                 |
| Wild specimens     | 54             | 12                  | 17                | 25                 |
| Fish meat pieces*  | 80             | 13                  | 27                | 40                 |
| Total              | 349            | 197                 | 69                | 83                 |

\*, three fish meat pieces, which identified as the Seven-band grouper, but were misidentified and mislabeled as the Longtooth grouper in two restaurants.

# MORPHOTYPES IN MALES CINNAMON RIVER PRAWNS *Macrobrachium acanthurus* (WIEGMANN, 1836)

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*Macrobrachium* male prawns often present morphotypes differentiation, which may influence farming management. We studied *Macrobrachium acanthurus* prawns from the Parnaíba River Delta, Northeast Region in Brazil, to verify the occurrence of morphotypes. Animals were weighted and analyzed according to color. Additionally, we measured body, chelipod parts and its angles. Then, animals were classified into classes of size, color and spination. Data were subjected to normality and homocedasticity tests prior to ANOVA. Multivariate (Principal Components) and relative growth analyses were also conducted. Results indicate that three distinct groups of males compose the population of this species of prawn: BLC (Blue Claw), BRC (Brown Claw) and TRC (Translucent Claw), which differ in morphology of chelipods and many morphometric relations.

Table 1. Mean  $\pm$  standard deviation of weight (W), total length (Lt), post-orbital length (Lpo), carapace length (Lcp), chelipod length (Lql), dactylus length (Ldc), merus length (Lm), carpus length (Lc), ischium length (Li), propodus length (Lpp); propodus width (Wpp); propodus angle (App), carpus angle (Ac), merus angle (Am) and ischium angle (Ai) of the three identified morphotypes of *M. acanthurus* males.

Table 1. Mean  $\pm$  standard deviation of weight (W), total length (Lt), post-orbital length (Lpo), carapace length (Lcp), chelipod length (Lql), dactylus length (Ldc), merus length (Lm), carpus length (Lc), ischium length (Li), propodus length (Lpp); propodus width (Wpp); propodus angle (App), carpus angle (Ac), merus angle (Am) and ischium angle (Ai) of the three identified morphotypes of *M. acanthurus* males.

|            | BLC                 | BRC                 | TRC                |
|------------|---------------------|---------------------|--------------------|
| <b>W</b>   | 10.67 $\pm$ 4.54 a  | 5.38 $\pm$ 1.95 b   | 2.46 $\pm$ 1.30 c  |
| <b>Lt</b>  | 89.03 $\pm$ 11.17a  | 72.13 $\pm$ 9.68 b  | 54.74 $\pm$ 9.19 c |
| <b>Lpo</b> | 73.33 $\pm$ 9.93 a  | 60.12 $\pm$ 6.97 b  | 46.15 $\pm$ 7.30 c |
| <b>Lcp</b> | 24.89 $\pm$ 3.68 a  | 20.07 $\pm$ 2.83 b  | 14.41 $\pm$ 2.84 c |
| <b>Lql</b> | 4.48 $\pm$ 0.28 a   | 3.99 $\pm$ 0.22 b   | 3.54 $\pm$ 0.21 c  |
| <b>Ldc</b> | 2.78 $\pm$ 0.30 a   | 2.20 $\pm$ 0.29 b   | 1.58 $\pm$ 0.32 c  |
| <b>Lpp</b> | 35.52 $\pm$ 10.02 a | 19.93 $\pm$ 5.95 b  | 10.56 $\pm$ 3.38 c |
| <b>Wpp</b> | 1.14 $\pm$ 0.29 a   | 0.78 $\pm$ 0.26 b   | 0.20 $\pm$ 0.31 c  |
| <b>Lc</b>  | 25.45 $\pm$ 7.56 a  | 15.27 $\pm$ 3.96 b  | 10.34 $\pm$ 1.88 c |
| <b>Lm</b>  | 19.94 $\pm$ 5.40 a  | 11.45 $\pm$ 3.23 b  | 7.40 $\pm$ 1.45 c  |
| <b>Li</b>  | 3.27 $\pm$ 0.30 a   | 2.76 $\pm$ 0.22 b   | 2.35 $\pm$ 0.23 c  |
| <b>App</b> | 3.96 $\pm$ 0.28 a   | 3.52 $\pm$ 0.29 b   | 3.33 $\pm$ 0.18 c  |
| <b>Ac</b>  | 59.00 $\pm$ 15.11 a | 31.46 $\pm$ 18.69 b | 9.70 $\pm$ 14.91c  |
| <b>Am</b>  | 4.07 $\pm$ 0.29 a   | 3.69 $\pm$ 0.25 b   | 3.62 $\pm$ 0.16 b  |
| <b>Ai</b>  | 54.99 $\pm$ 18.42 a | 17.03 $\pm$ 23.52 b | 0.36 $\pm$ 3.64 c  |

Means in the same line followed by different letters are significantly different ( $p < 0.05$ ).

## EFFECT OF BIOMASS DENSITY, HANDLING STRESS, AND NON-FOLLOWING OF SEDIMENT ON THE GROWTH AND SURVIVAL OF *Holothuria scabra*

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Trials were conducted over 6 months in 2016, in Tampilove, Madagascar, a site where *H. scabra* have been continuously farmed for over 7 years, to determine the effect of biomass density, handling stress and the number of years the sediment has been farmed, on the growth rate and survival of stocked animals.

Six replicate enclosures of 4 m<sup>2</sup>, stocked with 50 animals with a mean weight of 17 g (Range 11-23, SD=3.7), were built within farming pens that had been under continuous high density culture of *H. scabra* for periods of 7, 5, 3 and 0 years respectively, to determine if long term continuous culture affects the carrying capacity or the growth rates of *H. scabra* stocked within these potentially denuded sediments.

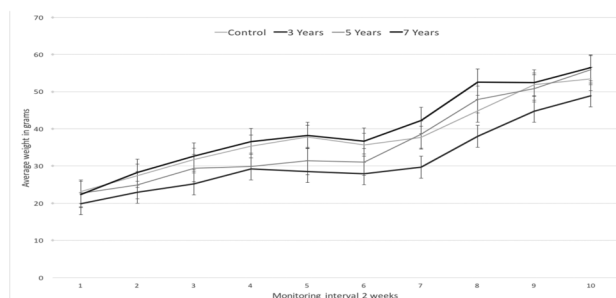
Stocked *H. scabra* were monitored fortnightly by collection and weighing with scientific scales, during the diurnal spring tide so as to ensure weights were not influenced by stomach contents. A second experiment trial sought to determine the effect this fortnightly monitoring had on the growth rate and survival of stocked *H. scabra*. Whilst a third sought to determine at what biomass density growth rates were retarded.

Results strongly indicate that successive years of high density culture does not significantly affect the carrying capacity of the farmed sediment, nor does it have significant effect on the growth rates or survival of *H. scabra*, within a period of 7 years.

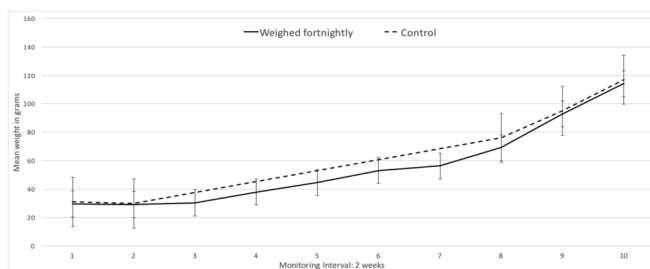
Handling stress of common monitoring methods were not shown to have a significant effect on *H. scabra* growth rates or survival. Increasing biomass density was shown to have a strong inverse correlation with growth and also indicates that growth rates are retarded at stocking densities far below the sediments *maximum* carrying capacity.

The research implies the use of fallow periods is not necessary to maintain high levels of growth within farmed sediments, that common monitoring practices do not negatively affect

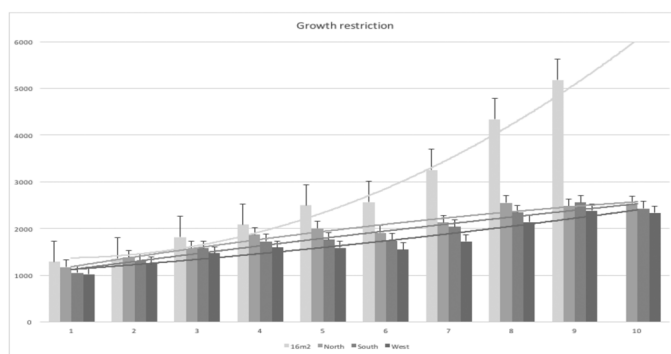
growth rate or survival, and that stocking strategies that result in a biomass density near the maximum carrying capacity result in stunted growth of farmed *H. scabra*. These results will be used to improve the farming practices and increase productivity and income generation potential of community-based farmers in Tampilove.



**Figure 1** Mean weight *H. scabra* stocked in pens with varying number of years under high density culture.



**Figure 2** Average weight of *H. scabra* that were monitored fortnightly, and control that were unmonitored four 5 months.



**Figure 3** Mean weights of *H. Scabra* in pens of varying stocking and biomass density



## FARMING MODEL CHANGES AND THEIR RATIONALE AFTER EXPERIMENTAL TRIALS AND 7 YEARS PROJECT HISTORY FARMING *Holothuria scabra* IN SEA PENS IN SOUTH-WEST MADAGASCAR

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Community-based farming of *H. scabra* in sea pens has shown to have considerable economic potential for communities along Madagascar's sheltered southwest coastline.

Marine conservation organization, Blue Ventures Conservation, has initiated and developed one of the longest running of these farming systems in the community of Tapolove, which has been under continuous production since 2009. Farmers report satisfaction with the project and over the course of the project's 7-year history, total sales have steadily increased and is now the main source of income for some community members. Due to an outbreak of Ulceration Syndrome in early 2015, the stocking of juvenile sea cucumbers was suspended. Blue Ventures took advantage of this latency period to thoroughly review the farming model and implement a number of experimental trials to inform the development of an improved model and best practice farming techniques. This presentation details the experimental trials that were undertaken to inform this development and other social lessons learnt over the project's history.

Experimental trials over 6 months in 2016 investigated the need for protective nurseries for juveniles <50 grams, the effect of handling stress on growth and survival, carrying capacity of the site sediment, whether the lack of fallow periods was limiting the potential growth or survival of stocked animals, and whether the addition of ground *Sargassum sp.* improved the growth rate. Also investigated was the growth of fast growing animals, or shooters, compared with the runs and therefore the potential for broodstock selection strategies to increase farm productivity.

Results of experimental trials indicated that:

- Protected nurseries did not significantly improve survival of stocked juveniles
- Handling stress did not significantly affect growth or survival.
- High potential to increase productivity through selection of shooters for broodstock.
- Addition of ground *Sargassum sp.* powder does not significantly increase growth.
- Non-fallowing of farmed sediment does not significantly alter its carrying capacity or the growth rates of stocked *H. scabra*.
- High survival >90 is achievable if theft can be deterred.
- Optimal sediment utilization can be achieved through continuous stocking and selective harvest practices.

A novel stocking strategy was developed to ensure maximum utilization of the sediments carrying capacity, whilst farm-lease systems were developed to ensure essential best practice aquaculture guidelines were adhered to by project participants. Increased security infrastructure and community security scheduling sought to ensure that chronic theft of mature animals was abated. Additional farm design improvements included the use of 316 stainless steel cable to hold farm netting in place and allowing pen netting to remain firmly fixed, allowing for easier cleaning and decreased amortization of capital infrastructural costs.

The results of these experimental trials and farming strategy improvements will be used to inform the development of an improved *H. scabra* farming model for communities in southwest Madagascar.

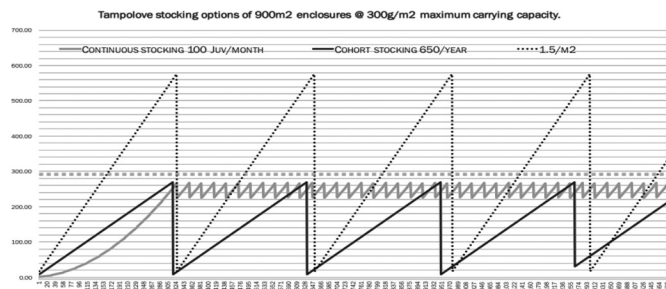


Figure 1 Various stocking strategies for Tapolove

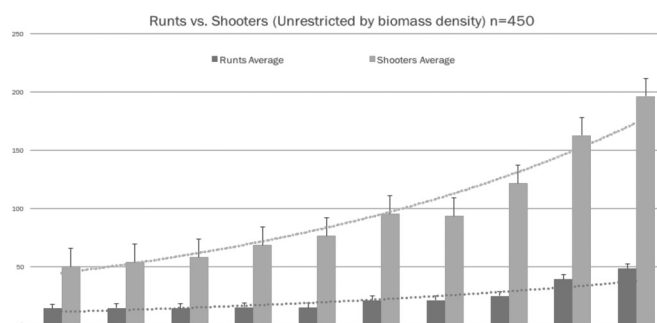


Figure 2 Runts vs. shooters: potential for genetic selection



## **PRACTICAL EXPERIENCE WITH THE DESIGN, CONSTRUCTION AND MANAGEMENT OF A COMMERCIAL INDOOR SUPER INTENSIVE SHRIMP CULTURE SYSTEM IN USA**

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The USA ranks as the third largest seafood consuming nation, and per capita shrimp consumption is 25% of all USA seafood consumed. (National Marine Fisheries Service 2015 ), and continues to rise. 30 years ago 80% of shrimp consumed in USA was from domestic sources, Today 90% is imported at a value of \$5 billion, only 10% from the USA both farmed and Wild sources. (USDA ERS 2016 ).

Over the past 20 years there have been many attempts in USA to culture shrimp commercially in a Re-circulating Aquaculture System. While the technological feasibility of growing shrimp in high density closed systems has been demonstrated, none of these ventures has been commercially successful. There are many reasons including the high energy costs required for the operation of multiple pumps, aerators, circulators and heaters, resulting in high production costs. These costs make it difficult to compete with the cheap imports.

The author has been involved in the design, construction and management of two indoor facilities over the past six years, one the largest of it's kind in the USA, and one his own private family in Florida. Results confirm the commercial viability of the system and through this presentation he will share his experiences, failures and success, and his future ideas and plans.

## FORMULATING TILAPIA DIETS ON DIGESTIBLE BASIS: A REVIEW OF PUBLISHED APPARENT DIGESTIBILITY COEFFICIENTS

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Precision feed formulation and feeding requires accurate knowledge of animal's nutrient requirements and of nutritional value of ingredients. An increased understanding of the nutritional requirements of tilapia, allowed the aquaculture industry to significantly reduce inclusion level of fish meal and, therefore, reduce feed and production costs. But while formulation on a digestible basis is for many years common practice in the poultry and swine industries, nutrient digestibility among ingredients and its variability within each ingredient are yet to be fully considered by the aquaculture industry. Several studies have been published in the past years on the nutrient digestibility of commonly used ingredients for tilapia but a comprehensive review of the available data is missing. The aim of our study was to review published apparent digestibility coefficients (ADC) of commonly used ingredients for Nile tilapia and perform a meta-analysis in order to better understand variability in ADC values. A total of 95 ingredients from 44 studies were included in a dataset comprising methodology employed as well as test ingredients nutrient composition and ADC. Most studies have reported ADC values for dry matter, crude protein (CP) and gross energy. At least 17 studies have published ADC for essential amino acids (EAA). Our review indicates that considerable differences exist in the methodology applied among studies. Less than half of the published data were considered as we excluded 27 studies that likely have produced erroneous values due to excessive leaching of nutrients or incorrect calculation of ADC.

TABLE1. Apparent digestibility coefficients (% , mean  $\pm$  SD) published in selected papers for fish meal (FM), meat and bone meal (MBM), soybean meal (SBM), cottonseed meal (CSM) and corn gluten meal (CGM) in tilapia. (n = number of ADC values considered for Crude Protein and AA ADC; \* only one value was available)

Our analysis shows that ingredients such as fishmeal, soybean meal and corn gluten meal are very well digested by tilapia with CP and EAA ADC values above 85% while other ingredients such as MBM and CSM show lower CP ADC (68.39 and 79.37% respectively). For some ingredients such as MBM, differences in ingredient quality are likely to explain the large variability in ADC. Data also show that ADC of individual EAA does not always correspond to that of CP, suggesting the importance of knowing ADC values for each of the EAA. Finally, we have also performed regression analysis to understand how experimental factors and nutrient levels affect nutrient digestibility. Findings of this review will be highly valuable for commercial feed producers to formulate tilapia diets on a digestible basis.

TABLE1. Apparent digestibility coefficients (% , mean  $\pm$  SD) published in selected papers for fish meal (FM), meat and bone meal (MBM), soybean meal (SBM), cottonseed meal (CSM) and corn gluten meal (CGM) in tilapia. (n = number of ADC values considered for Crude Protein and AA ADC; \* only one value was available)

| <b>Ingredient</b> |     | <b>Dry</b>        | <b>Crude</b>      | <b>Gross</b>     | <b>Lysine</b>     | <b>Methionine</b> | <b>Threonine</b>  |
|-------------------|-----|-------------------|-------------------|------------------|-------------------|-------------------|-------------------|
| <b>(n)</b>        |     | <b>Matter</b>     | <b>Protein</b>    | <b>Energy</b>    |                   |                   |                   |
| <b>FM</b>         | (5) | 91.54 $\pm$ 3.79  | 90.93 $\pm$ 4.87  | 93.71 $\pm$ 3.26 | 93.43 $\pm$ 3.91  | 92.69 $\pm$ 4.31  | 90.83 $\pm$ 4.12  |
| <b>MBM</b>        | (7) | 57.73 $\pm$ 13.90 | 68.39 $\pm$ 12.81 | 79.38 $\pm$ 6.70 | 80.29 $\pm$ 7.75  | 83.17 $\pm$ 8.41  | 81.97 $\pm$ 12.05 |
| <b>SBM</b>        | (8) | 82.80 $\pm$ 7.71  | 94.25 $\pm$ 3.88  | 85.20 $\pm$ 4.51 | 94.13 $\pm$ 4.82  | 93.03 $\pm$ 5.69  | 91.62 $\pm$ 4.34  |
| <b>CSM</b>        | (3) | 65.90*            | 79.87 $\pm$ 1.70  | 78.90*           | 77.73 $\pm$ 10.94 | 83.03 $\pm$ 10.58 | 68.80 $\pm$ 14.17 |
| <b>CGM</b>        | (5) | 88.20 $\pm$ 8.58  | 91.02 $\pm$ 5.55  | 88.08 $\pm$ 5.47 | 91.67 $\pm$ 4.34  | 94.79 $\pm$ 4.40  | 88.95 $\pm$ 6.92  |

## **AQUACULTURE FOOD SAFETY AND DISEASES...PREVENTATIVE CONTROLS FOR AQUACULTURE**

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Aquaculture is the primary component of the seafood trade which makes up the largest traded food group in the world. More than all grains, banana, cocoa, rice, and all other meats combined. Aquaculture products are also the primary source of protein for one fifth of the world's population, mainly in the developing world. This is extremely important for food security because the world's population is expected to grow from 6.8 to 9.2 billion in 2050, causing the demand for food to increase 110%. This will result in a huge demand...an opportunity... for aquaculture products. However, there are serious food safety concerns with aquacultured products. These concerns include the presence of unsafe residues, pathogens, and the potential to contribute to antimicrobial resistance. When food safety problems are found there are a range of negative impacts. These include significant costs to producers, processors, buyers, importers, and the regulatory agencies, market disruptions, an increase in the negative perception of aquaculture, lost markets, etc. This talk will describe a new way of approaching aquaculture food safety, minimizing the risk of trade interruptions due to food safety concerns, and an approach to obtaining more customer confidence in aquaculture.

## **THE RESEARCH OF THE EFFECTS OF QUINOA's (*Chenopodium quinoa*) AS EDIBLE BIOFILM ON THE RAINBOW TROUT's (*Oncorhynchus mykiss*) FILLETS SHELF-LIFE**

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Along with the changing consumer demand, researches on food packaging has increased, particularly known as environmental implications and non-negative in terms of health, interest on biofilms has increased. In this study, structural features (thickness and light transmittance) of edible films obtained from quinoa starch are identified and obtained films are applied on rainbow trout's fillets (*Oncorhynchus mykiss*). Storage period at a low temperature ( $4 \pm 1$  °C), trout fillets covered with quinoa biofilm were subjected to microbiological (total aerobic mesophilic bacteria, lactic acid bacteria, *Pseudomonas* ve *Enterobacteriaceae*) and chemical (TVB-N, TBARS and pH) analysis in predetermined days (0, 3, 6, 9 ve 12) during shelf life. Our result showed that film thickness is  $0.195 \pm 0.010$  mm and light transmittance values in different wavelengths are measured between 10-37%. Storage at low temperature in trout fillets belonged to different treatment (control and film) TVB-N, TBARS and the pH values and in the numbers of total aerobic mesophilic and psychotropic, lactic acid bacteria, *Pseudomonas* and *Enterobacteriaceae* statistical differences are determined ( $p < 0.01$ ,  $p < 0.05$ ). As a conclusion, the films obtained from quinoa starch are observed to have chemically and microbiologically protective effect in trout's fillets during storage at +4 °C temperature.

## **AQUACULTURE POLICIES, AGRICULTURAL TRADITION AND PISCICULTURE DEVELOPMENT IN KORHOGO DEPARTMENT (COTE D'IVOIRE)**

KOUADIO Nanan Kouamé Félix

Department of Geography, Peleforo Gon Coulibaly University (Ivory Coast)

Like the other regions of Côte d'Ivoire, the Korhogo department in the north of the country has benefited from the aquaculture policies put in place by the Ivorian government since the early 1960s and which have This area through the realization of multiple projects between 1977 and 1985 (ASSI-KAUDJHIS, 2011). However, unlike the south of the country, activity is waning in the north especially in the department of Korhogo. It is trampling to such an extent that its socio-economic and spatial footprints today are clearly less perceptible. By way of illustration, it can be noted that in 2014, the former savannah region of which Korhogo was the chief town, had only 02 fish farmers out of 1,369 nationally, or about 0.15%. There were also 70 ponds out of a national total of 5 623, equivalent to a contribution rate of 1.24% (National Association of Aquaculture Farmers of Côte d'Ivoire: ANAQUACI, 2014). Yet, the department of Korhogo is a real "labor pool" (HAUHOOT A., 1979). It has an important human capital, based on the reputation of the Senoufo people, presented as a "courageous, laborious, loving, and passionate people" (M'BRAH D., 2013). In connection with the aquaculture policies initiated by the Ivorian administration and the agricultural tradition of the populations, the study questions the level of development of fish farming in the department of Korhogo.

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At the level of the results, the analysis of the development policy of aquaculture attests to a plurality of actions in this direction before and after the independence of Côte d'Ivoire. This policy initiated by the colonizer was reinforced in 1960 by the Ivorian State, the majority of whose development projects were carried out in the north of the country, notably in the department of Korhogo. However, given the lack of an aquaculture tradition in spite of the existence of an agricultural culture of the populations, these actions have not had the expected results. Analysis of the aquaculture landscape in our study area attests to a past-lived aquaculture. We note the occupation of its production spaces by other agricultural activities including rice cultivation, many abandonment of farms and few functional ponds and the decline of local production of farmed fish.

## **AQUACULTURE POLICIES, AGRICULTURAL TRADITION AND PISCICULTURE DEVELOPMENT IN KORHOGO DEPARTMENT (IVORY COAST)**

KOUADIO Nanan Kouamé Félix

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Like the other regions of Côte d'Ivoire, the Korhogo department in the north of the country has benefited from the aquaculture policies put in place by the Ivorian government since the early 1960s and which have This area through the realization of multiple projects between 1977 and 1985 (ASSI-KAUDJHIS, 2011). However, unlike the south of the country, activity is waning in the north especially in the department of Korhogo. It is trampling to such an extent that its socio-economic and spatial footprints today are clearly less perceptible. By way of illustration, it can be noted that in 2014, the former savannah region of which Korhogo was the chief town, had only 02 fish farmers out of 1,369 nationally, or about 0.15%. There were also 70 ponds out of a national total of 5 623, equivalent to a contribution rate of 1.24% (National Association of Aquaculture Farmers of Côte d'Ivoire: ANAQUACI, 2014). Yet, the department of Korhogo is a real "labor pool" (HAUHOUOT A., 1979). It has an important human capital, based on the reputation of the Senoufo people, presented as a "courageous, laborious, loving, and passionate people" (M'BRAH D., 2013). In connection with the aquaculture policies initiated by the Ivorian administration and the agricultural tradition of the populations, the study questions the level of development of fish farming in the department of Korhogo.

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## LARGE SCALE HIGH-RATE BIOFLOC TANK CULTURE OF TILAPIA IN MALAWI: FROM INCEPTION TO TECHNICAL SUCCESS

Ramon M. Kourie\* and Bryce Fleming

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In 2006 SustAqua Fish Farms (Pty) Ltd was commissioned by Chambo Fisheries to design a large-scale Biofloc Technology (BFT) fish farm on the outskirts of Blantyre, Malawi based upon the use of feral tilapia species *Oreochromis mossambicus* and *Oreochromis shiranus*. It was only in September 2013 that the project infrastructure was ready to support production in four of eight 766m<sup>3</sup> sequentially managed continuous BFT grow-out tanks of a proprietary design.

Following the implementation of bioenergetic feeding rate models average feed conversion ratio's of 1 : 1 on 20.2% protein feed (providing a C: N ratio of approximately 15.5:1) were routinely achieved under large scale culture conditions. The calculated contribution of filter feeding on biofloc by *O. mossambicus* and *O. shiranus* falls in the range of 20-25% of daily Digestible Energy (DE) requirements for fish in the size range 20-220g. Net Protein Retention (NPR) and Net Energy Retention (NER) values were greater than 75% and 40% respectively significantly higher than a typical commercial Recirculated Aquaculture System (RAS), lake cage culture and greenwater pond farming operations. These results would suggest that properly designed and managed biofloc tank culture of tilapias is potentially the most efficient form of feedlot livestock production outperforming lamb, broiler chickens, pigs and beef steers as well as Atlantic salmon in net-pens and tilapia under typical lake cage culture, greenwater pond farming and RAS conditions in terms of protein recovery on an edible meat yield basis.

These results, notably significant feed cost savings, high annual production yields (attributed to the use of a sequential production schedule) coupled with the efficient use of power and capital employed, year-round production potential (under greenhouse enclosures where necessary) and good fish flavour quality offers an interesting new dimension for tilapia aquaculture development on the periphery of major African cities on the continent.

## **PRACTICAL DAILY FEED ALLOTMENT ESTIMATES IN THE CULTURE OF FERAL TILAPIA SPECIES *Oreochromis shiranus* AND *Oreochromis mossambicus* UNDER LARGE SCALE HIGH-RATE BIOFLOC TANK CULTURE CONDITIONS IN MALAWI**

Ramon M. Kourie\* and Bryce Fleming

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Estimating optimum feeding rates under practical commercial conditions in tilapia culture systems remains an enigma to most fish farmers. A simplified method of estimating daily energy requirements of the fish reared in tilapia culture systems is presented which requires data normally routinely captured by fish farmers including the daily increment of weight gain, the energy content of the feed fed, individual average body weight and water temperature data. The outputs include an estimate of the Digestible Energy Need (DEN) in units of Mega Joules of Digestible Energy to yield 1kg of weight gain and the required daily energy requirements in a cohort or population of fish (DE, MJ/kg of fish day<sup>-1</sup>), the required feeding rate in absolute terms expressed as the daily Feed Allotment (dFA) and lastly feeding rates expressed as a percentage of body weight per day (% BW/day<sup>-1</sup>) in a cohort or population of fish.

Following inventory taking under large-scale Biofloc Technology (BFT) conditions at Chambo Fisheries in Malawi a bioenergetic feeding rate model was constructed, building onto the basic feeding rate model, to include the contribution of biofloc grazing toward meeting a portion of the digestible energy requirements of *O. mossambicus* and *O. shiranus* under farm conditions. Bioenergetic feeding rate models have never been properly applied to biofloc tank culture of tilapia anywhere in the world and published process optimization studies are still pending by the global research community (Avnimelech, 2007). This work then represents first attempts to optimize feeding rates counting the contribution of biofloc grazing towards meeting a portion of the digestible energy requirements of the fish reared under commercial large-scale BFT conditions.



## **ECONOMICS, SUSTAINABILITY AND RISKS FACTORS ASSOCIATED WITH LARGE SCALE COMMERCIAL TILAPIA CULTURE IN MALAWI: A COMPARISON OF PRODUCTION SYSTEMS**

Ramon M. Kourie\* and Bryce Fleming

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Few economic studies providing detailed economic break-downs of capital investment, operating costs and returns in large-scale commercial tilapia culture systems in Africa exist. Lutz (2000) produced one of the only published accounts on the economics and potential competitive dynamics of commercial tilapia aquaculture operations scaled for owner-operators in the Americas in the literature.

In addition, studies are lacking on the economics, sustainability and risk factors of Biofloc Technology (BFT) systems under commercial conditions. Based upon empirical data captured from Chambo Fisheries in Malawi, purportedly the largest tank farm in Africa and the largest tilapia biofloc tank farm in the world, an economic model has been constructed comparing investment and farm gate production costs across four major tilapia farming grow-out technologies all producing 800 tonnes per annum (live weight) given placement on or near Lake Malawi.

The study indicated that farm gate production costs on a live weight fish basis were US\$ 2.09/kg for the Lake Cage Culture model, US\$1.31/kg for the SAFF-BFT farm, US\$1.42/kg for the Greenwater Pond farm and US\$1.75/kg for the SAFF-Recirculated Aquaculture System (RAS) farm. The production cost advantage of the SAFF-BFT was significant at 59.5% over the lake cage culture model, 8.35% over the Greenwater Pond farming model and 33.6% over the SAFF-RAS operation.

Sustainability indicators were assessed for the four commercial tilapia farming operations where the SAFF-BFT and SAFF-RAS farms performed best followed by the Greenwater Pond farm. The large-scale Lake Cage Culture Farm was considered unsustainable based upon high nitrogen and phosphorus emissions and Total Fecal Solids (TFS) loading into lake ecosystems. Furthermore, the large-scale lake cage culture model fails to achieve economic sustainability at selling prices competitive with the lowest cost supply sources of wild-caught fish protein imported from Namibia and South Africa which retails at US\$2.20/kg in neighbouring Mozambique. Among the risk factors highlighted stock theft was identified as the major risk factor impacting profitability particularly in the Lake Cage Culture and Greenwater Pond Farming operations due to greater difficulties to implement effective security measures in practice in Malawi.

## MULTIDIMENSIONAL MODELS OF SURVIVAL OF FISH FINGERLINGS AND FORMATION OF FISH PRODUCTIVITY OF MATURING PONDS

Aleksandr Kozlov<sup>a</sup>, Vitold Pestis<sup>a</sup>, Tamara Kozlova<sup>a\*</sup>, Inna Lojko<sup>a</sup>, Natalya Dmitrovich<sup>b</sup>, Juri Goncharik<sup>c</sup>

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Multifactor modeling allows determining the optimal parameters of intensification methods of stimulating the natural food supply in order to increase the bio-productivity of pond ecosystems.

The results of seven-year studies of the hydrobiological regime of fish ponds and the study of the influence of abiotic and biotic factors on the formation of productivity of maturing ponds are given in conditions of intensive fish breeding.

Multidimensional-multifactorial models of survival of fingerlings (%) and fish productivity (c/ha) were constructed depending on a number of factors.

The correlation model of fingerlings survival was as follows:

$$Y_x = -99.8 + 9.25X_1 - 0.139X_2 + 0.0004X_7 + 0.061X_{13} - 0.128X_{15} - 0.195X_{17} - 0.57X_{19} + 0.58X_{22} - 0.37X_{23} - 0.57X_{24}, \quad (R = 0.726, F_1 = 4.47).$$

The increase in fingerlings survival occurs with an increase the water temperature to 22.4°C ( $X_1$ ), the consumption of mixed fodder ( $X_7$ ), the increase in the zooplankton biomass ( $X_{13}$ ), and the increase in the degree of overgrowing of pond by macrophytes ( $X_{22}$ ). The decrease in fingerlings survival occurs with an increase in planting rate of carp larvae ( $X_{15}$ ), silver carp ( $X_{17}$ ) and bigmouth buffalo ( $X_{19}$ ), and also with an increase in the pond area ( $X_{24}$ ).

The correlation model of fish productivity was as follows:

$$Y_x = -4.96 + 0.96X_1 + 0.0015X_7 - 0.134X_{10} + 0.029X_{16} + 0.861X_{18} + 0.044X_{13} + 0.61X_{20} - 0.322X_{22} + 0.23324 - 10.03X_{25}, \quad (R = 0.801, F_1 = 7.05).$$

With an increase in the degree of overgrowing macrophytes ( $X_{22}$ ) to 32.6%, fish productivity decreases. The increase in the consumption of mixed fodder ( $X_7$ ), the planting rate of grass carp ( $X_{16}$ ), silver carp ( $X_{18}$ ), and the planting rate of uncharted fish larvae contributed to the growth of fish productivity.

Increasing the fingerlings survival and increasing the fish productivity of ponds require different approaches and require different requirements for the most important biological parameters. If the increase in the survival rate of the fingerlings increases with a decrease in the area of the pond and an increase in their overgrowth, the increase in fish productivity is associated with an increase in the pond area and a decrease in their overgrowth. A positive effect on the growth of fish productivity and survival of fingerlings is the increase in the planting rate of silver carp and spotted silver carp.

## SUSTAINABLE GOVERNANCE OF OFFSHORE AQUACULTURE IN ISRAEL

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Currently, no policy framework, impact assessment or long-term monitoring program is set for the expansion of offshore aquaculture industry in Israel. This Ph.D. research will combine mixed methods in order to determine the most effective policy route for sustainably governing the aquaculture industry in Israel. Relevant international, regional and national legislative instruments and agreements that govern this sector will be analyzed with a specific focus on environmental protection. This review will concern all countries with prepared or active policies regarding offshore aquaculture, and will be assessed using thematic analysis according to criteria/parameters applicable in the governance of this industry. Discrepancies within the environmental policies will be defined through gap analysis, and include the relative advantages and disadvantages of development, sensitive to cultural situation.

An analytical approach to Israel's main environmental legal and scientific instruments will be applied to determine knowledge and efficiency gaps. Various stakeholders will be interviewed to garner their perceptions on developing aquaculture in Israel, with particular focus on decision-making and participation. This research will determine what types of information are necessary for policy development in aquaculture, find the gaps in ecological prioritization and define the socio-ecological limitations (if any) existing for the development of aquaculture in Israel.

Using the conclusions from the global analysis, stakeholder insights, ecosystem modelling, and risk assessments, I will model various policy scenarios to qualitatively analyze the institutional and legal basis of aquaculture for the Israeli EEZ. This analysis hopes to define the appropriate spatial-temporal management approach using Ecopath with Ecosim and MARXAN software. Applying the ideology of adaptive governance within a participatory multi-criteria framework of decision support tools will provide an efficient yet flexible approach capable of bridging the gap between science and policy.

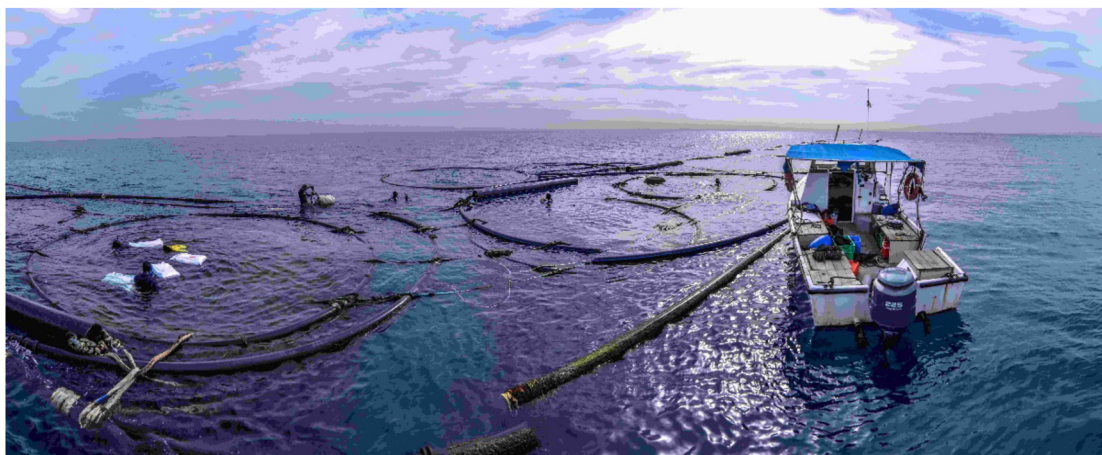


Figure 1. SUBflex marine cages offshore of Ashdod, Israel

(photo credit: Aviram Valdman)

## EFFECT OF EARLY SIZE SORTING ON CANNIVALISM AND SURVIVAL IN EUROPEAN PERCH *Perca fluviatilis* LARVAE

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Intra cohort cannibalism is one of the main factors that affect the survival of cultured fishes, especially in the larval and juvenile stages. High size variation of the fish can facilitate the emergence of cannibalism. Size sorting is a common procedure used during intensive juvenile fish rearing to reduce size variations in the cohort and to mitigate the impact of cannibalism.

In the literature studies that focus on the effects of size sorting on perch larvae are missing. Therefore, the present study was designed to investigate the effect of size sorting on survival and cannibalism in European perch larvae under controlled conditions.

Two weeks experiment was conducted with perch larvae aged 34 days post-hatch. Three experimental groups in triplicate were conducted – sorted small specimens (group S – average body weight 0.06 g), sorted large specimens (group L – average body weight 0.15 g), and unsorted ones (group U – average body weight 0.10 g). Larvae were reared in tanks with a volume of 15 L each, set in recirculating system at 21 °C. In each group, the stocking density was 5 larvae L<sup>-1</sup>. During experiment perch were feed with commercial diet in *ad libitum*. Dead larvae were removed daily and were classified as victims of type I cannibalism (posterior damaged or tailed-off) and others dead (starving, intact). All individuals were counted at the end of the experiment and the difference between the initial number of larvae and final number of alive and dead larvae were considered as victims of type II cannibalism (number of lacking fish). The data were analyzed using one-way ANOVA. Following validation of the normal distribution of the data, post hoc LSD Fisher test was used ( $P < 0.05$ ).

The significant lowest losses to type I cannibalism in both sorted groups were observed, 4.3 and 3.2% for S and L, respectively (Fig. 1). In unsorted group (U) losses to type I cannibalism exceed 18%. Losses to type II cannibalism ranged from 2.2 to 4.9 %, but not differ significantly between groups. At the end of the experiment survival in both sorted groups exceed 92% and were significant higher than in unsorted ones (75.6%).

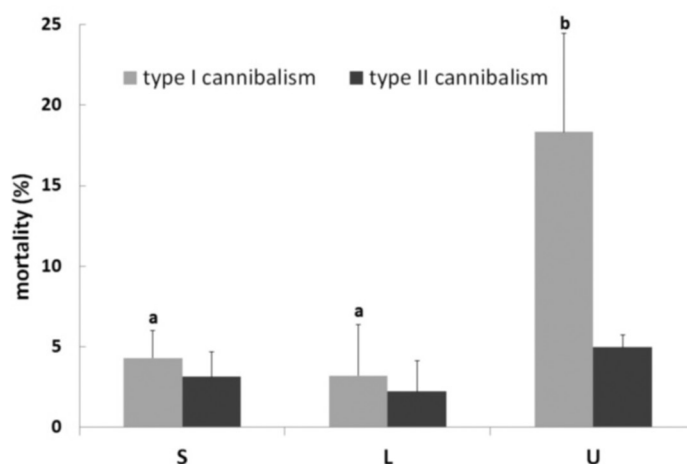


Fig. 1 Respective contributions of type I and type II cannibalism, in the experimental groups of perch larvae.

## ALTERNATIVE PROTEIN SOURCES TO REPLACE *Rastrineobola argentea* FISHMEAL FROM AQUAFEEDS IN EASTERN AFRICA

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Fishmeal (FM), the ideal source of protein for most fish species is increasingly becoming less available for aquafeed production. In E. Africa, the silver cyprinid, *Rastrineobola argentea*, a pelagic fish is the main source of FM used in fish feeds. However, the high demand for *R. argentea* for livestock feeds and human food has escalated the prices and made it less available for aquafeed production. Therefore, there is a need to search for locally accessible protein sources that are in limited or not in use. Agricultural and freshwater based non-conventional protein sources were examined for feed formulation and production in Uganda.

The protein and essential amino acid (EAA) composition of *Rastrineobola argentea*, the freshwater shrimp (*Caridina nilotica*), mung beans (*Vigna radiata*) and two freshwater bivalves: *Coelatura cf. Cridlandi* and *Mutela cf. bourguignaiti* were analyzed following AOAC, (1995). A growth study was done with juvenile Nile tilapia to further assess their suitability (results not shown).

Table 1. Crude protein (g/kg) and essential amino acid content (g/kg) of conventional and non conventional protein sources in Uganda. Values in parentheses show the proportion of each EAA to the total EAA within each raw material source.

Table 1. Crude protein (g/kg) and essential amino acid content (g/kg) of conventional and non conventional protein sources in Uganda. Values in parentheses show the proportion of each EAA to the total EAA within each raw material source.

| E-amino acid  | <i>R. argentea</i> | <i>C. nilotica</i> | <i>V. radiata</i> | <i>C. cridlandi</i> | <i>M. bourguignaiti</i> |
|---------------|--------------------|--------------------|-------------------|---------------------|-------------------------|
| Crude protein | 530-600            | 650                | 238               | 635                 | 642                     |
| Arginine      | 53.5 (16)          | 55.7 (21)          | 20.2 (15)         | 32 (15)             | 38 (16)                 |
| Histidine     | 23 (7)             | 14.2 (5)           | 9.9 (7)           | 8 (4)               | 12 (5)                  |
| Isoleucine    | 29 (9)             | 24.5 (9)           | 11.8 (9)          | 20 (10)             | 25 (10)                 |
| Leucine       | 52.9 (16)          | 42.3 (16)          | 22.6 (17)         | 38 (18)             | 43 (18)                 |
| Lysine        | 62.2 (19)          | 43.2 (16)          | 22.3 (16)         | 31 (15)             | 34 (14)                 |
| Methionine    | 14.8 (4)           | 10.9 (4)           | 4 (3)             | 11 (5)              | 11 (5)                  |
| Phenylalanine | 29.4 (9)           | 24.9 (9)           | 17.3 (13)         | 19 (9)              | 25 (10)                 |
| Threonine     | 29.8 (9)           | 22.3 (8)           | 10.2 (7)          | 22 (11)             | 25 (10)                 |
| Tryptophan    | 6.1 (2)            | 5.6 (2)            | 2.8 (2)           | 4 (2)               | 4 (2)                   |
| Valine        | 32.9 (10)          | 27.2 (10)          | 15.2 (11)         | 24 (11)             | 27 (11)                 |

The protein content of *V. radiata* was only about a third of the protein content of *C. nilotica*, *C. cridlandi* and *M. bourguignaiti*; and about half of *R. argentea*. As a result the essential amino acids (EAA) content is lower in *V. radiata* (Table 1) than in the *R. argentea*, *C. nilotica* and the bivalves. The proportion of EAA in *C. nilotica*, *C. cridlandi* and *M. bourguignaiti* are comparable to that of *R. argentea* fishmeal. The results suggest that *C. nilotica* and the bivalves studied could replace *R. argentea* from fish feeds as protein sources

**DETECTING THERMAL-SHOCK AND-ACCLIMATION IN THE SEA CUCUMBER *Holothuria scabra*, USING A MULTIPLE BIOMARKER APPROACH**

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Water temperature is among the most important environmental variables determining production efficiency in sea cucumber aquaculture. Ectothermic sea cucumbers are affected by temperature changes at virtually all levels of biological organization, ranging from gene expression and enzyme activities, at the molecular and biochemical levels, to energy metabolism and growth, at the organism level. Despite the commercial and ecological relevance of sea cucumbers, knowledge about their physiological- and cellular stress responses to various abiotic factors, such as temperature, is still scarce.

In this study, a multiple biomarker approach was established for *H. scabra*, to generate a holistic picture of molecular, biochemical and energetic processes, which may help to determine thermal acclimation capacities as well as threshold levels to acute temperature shocks. The selected biomarkers comprise; gene expression analyses of HSP70 (RT-qPCR); cellular energy consumption (ETS activity); energy related enzymes' activity (IDH and LDH); quantification of energy reserves (lipids, carbohydrates and proteins); and whole-organism respiration rate (see Fig.1). These biomarkers were applied in two different experimental scenarios. 1) Acclimation to sub- lethal temperature: Exposure of *H. scabra* to cold (21°C) and warm (33°C) temperature conditions, over a period of 30 days. 2) Maximum and minimum temperature threshold levels: Exposure of *H. scabra* to rapid temperature changes ( $\pm 2^{\circ}\text{C}/\text{h}$ ), in order to create cold (max. low temperature 17°C) and warm (max. high temperature 41°C) temperature shocks.

The results presented provide novel insights into thermal-stress physiology of *H. scabra* crucial for the development of optimal rearing conditions, as well as for identifying reliable health markers for cultured and free-living sea cucumbers.

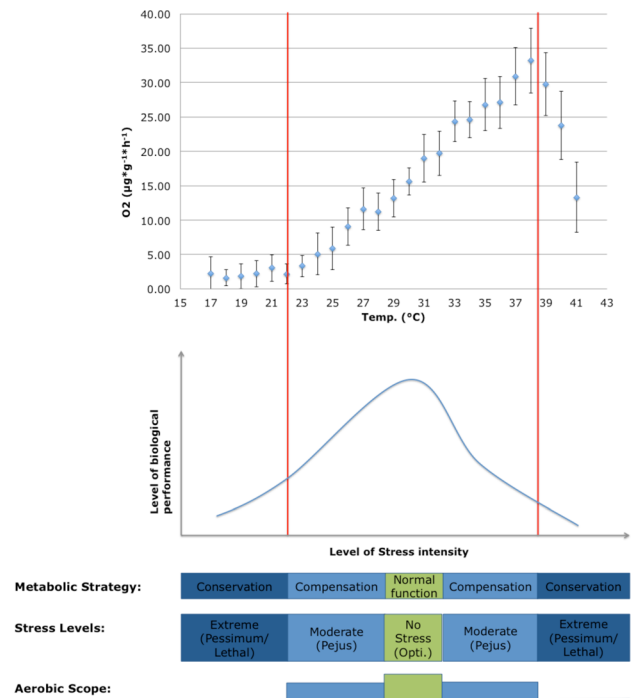


Fig. 1: Data points represent means (n=8) and standard deviation of respiration rates of *H. scabra* at different temperature levels. The peak of the bell shaped curve below, depicts energy balance at optimal temperature. Above or below optimal temperature, *H. scabra* suffers moderate stress (pejus range, with reduced aerobic scope). When a critical high or critical low temperature is reached (vertical red lines), the organism suffers extreme stress (pessimum to lethal range, with diminished aerobic scope)



## REARING OF RIVER LAMPREY *Lampetra fluviatilis* LARVAE FED WITH *Artemia* spp. UNDER CONTROLLED CONDITIONS

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River lamprey *Lampetra fluviatilis* (L.) belongs to aquatic organisms, which population has declined to a significant extent, particularly in N Europe. Due to pollution, river engineering and various impassable barriers (weirs, dams, etc.) a lot of individuals cannot reach to the spawning grounds. Because of its serious decline, the river lamprey is now given some protection being listed in annexes IIa and Va of the EU Habitats Directive 92/43/EEC or recorded as VU (*Vulnerable*) in the Polish Red Book of Animals. The lack of information about its ecology and distribution poses difficulties to the identification of specific action directed to its conservation. To avoid the deterioration of lamprey's status to EN – *endangered* species, or even to CR – *critically endangered* species, an effective conservation programmes considering the spawning capability followed by the larvae rearing in controlled conditions are required. Considering that the river lamprey spawns as seldom as only once in its life cycle, a best-practice monitoring protocol for lamprey should be developed, as well.

River lamprey is a representative of parasitic and anadromous jawless species – the most primitive of all living vertebrates. Its larvae, called ammocoetes, contrary to fish larvae, do not swim but remain burrowed in the sand-gravel substrate waiting for drifting food as fine particulate matter, mainly micro-organisms such as desmids and diatoms. Thus, it is important to provide them substrate in which young larvae are able to burrow themselves and be protected against the water flow.

In our study we used spawners of river lamprey obtained from the Vistula Lagoon (SE Baltic Sea, Poland). Gametes were obtained and fertilized under controlled conditions. Afterwards the spawn was incubated in Weiss mini-jars in water at 16°C. Before taking the external food, the hatched larvae resorb the yolk. After nearly completion of yolk sac resorption, lamprey larvae at a length of 9.1 mm ( $\pm 0.12$ ) were placed in 3 flow-through fish-tanks with the bottom areas as big as 0.3 m<sup>2</sup> covered by coarse grained sand of  $\varnothing = 0.3 - 0.8$  mm and water depths of 10 cm. Water flow was maintained at 0.5 dm<sup>3</sup>/min. The larvae density amounted to 100 individuals/dm<sup>2</sup> of the bottom area. The sand was placed on the filtering platform to ensure optimal water circulation and aeration conditions for larvae. The thickness of substratum was 1 cm. The water temperature during the rearing was 20.0 $\pm$ 0.5°C. The larvae, from the very beginning, were fed with live *Artemia* nauplii, at the 1<sup>st</sup> larval stage (<450  $\mu$ m in length). Live *Artemia* nauplii, was rinsed in clean freshwater, and then was given every 4 hours during the daytime. As a result of 3 weeks of rearing, an average biomass of river lamprey larvae amounted to 23.8 mg ( $\pm 4.22$ ), while body length amounted to 18.2 mm ( $\pm 1.43$ ). The survival rate of larvae reached 67%. The developed method of lamprey rearing may contribute to successful maintaining and reinforcement of the lamprey local populations endangered of extinction, and even to their restitution.

The project was supported by National Science Center in Poland within a grant No DEC-2013/09/B/NZ9/03130.



# TOWARDS THE DEVELOPMENT OF A VACCINE AGAINST LACTOCOCCOSIS IN FARMED RAINBOW TROUT

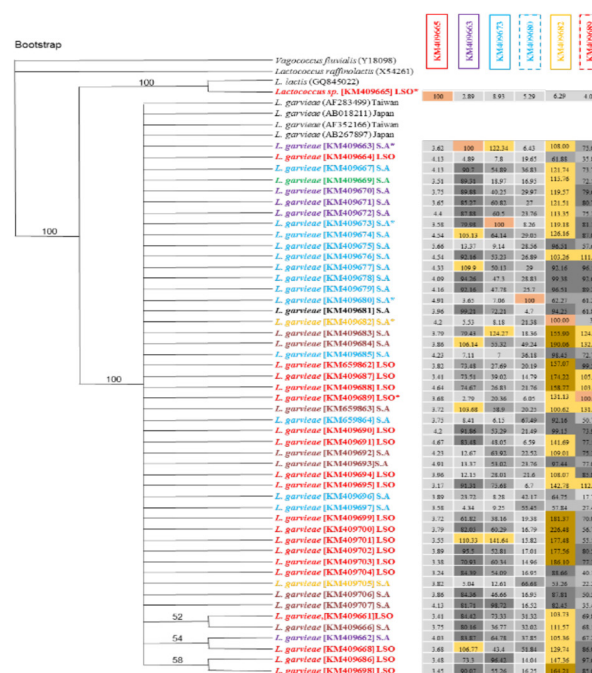
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Vaccination is often regarded as the most feasible means of controlling bacterial disease and has been used extremely successfully within the aquaculture industry. However, the efficacy of many developed vaccines is often poor. Proper identification of pathogenic bacteria, including the main virulence factors towards their pathogenicity, is essential information for the development of vaccines with lasting efficacy.

*Lactococcus garvieae*, the aetiological agent of Lactococcosis, has been responsible for periodic disease outbreaks on rainbow trout (*Oncorhynchus mykiss*) farms in South Africa and Lesotho since the late 1970s. Pathogenic traits expressed by *L. garvieae* are however not fully understood, and, to date, vaccines that have been created from some of the isolates from the region have lacked lasting efficacy. Without proper knowledge of the different serotypes that exist among these *L. garvieae* isolates, which is information crucial for development of vaccines with high efficacy, lactococcosis will continue to cause problems for the rainbow trout industry.

In this study, a total of 50 *Lactococcus* isolates (49 *L. garvieae* and 1 *Lactococcus* sp.), that had been isolated from 2006-2012 from diseased rainbow trout farmed in Lesotho (n=22) and South Africa (n=33), were characterized genetically and antigenically. Genetic analysis revealed vast intraspecies diversity among *L. garvieae* strains isolated from both countries. Antigenic characterization was performed, with six rabbit produced polyclonal antibodies using an Enzyme Linked-Immunosorbent Assay (ELISA), to improve our understanding of the clonality of the *L. garvieae* isolated from different localities. Our results suggest that more than one serotype of *L. garvieae* exist within the population, suggesting high antigenic diversity among the isolates and a possible reason for the ineffectiveness of vaccines produced to date. Of the six polyclonal antibodies tested, antibodies against isolate KM409682 showed the highest cross reactivity among the isolates, suggesting that it may be a good candidate for vaccine development. Our data highlights the importance of antigenic characterization for the design and development of new vaccines and will contribute towards the development of an effective vaccine(s) for farmed rainbow trout in southern Africa.



**Figure 1:** (A) Bootstrap phylogenetic tree derived from parsimony analysis of the 16S rRNA gene sequence of 49 *L. garvieae* and 1 *Lactococcus* sp. Included are ELISA results obtained from antigenicity analysis. The numbers are percentages of reactivity and coloured boxes relate to the intensity of reactivity: <20 %; 20- 49 %; 50- 79 %; 80- 99 %; 100- 149 % and >150 %.

## RAPID AND ASSISTED MULTIPLICATION TECHNIQUE OF AFRICAN CATFISH *Clarias gariepinus*

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To meet the challenge of malnutrition, food, poverty and food insecurity on a global scale, particularly in sub-Saharan Africa, aquaculture is proving to be a significant asset. Among aquaculture breeding species that deserve sustained attention, there is the African Catfish.

African catfish accounts for 80% of aquaculture production in some countries in sub-Saharan Africa, including Nigeria. In the Democratic Republic of Congo, 59% of fish farmers in western DRC raise African catfish, usually in combination with tilapia.

In many of the fish farms in Central Africa, African catfish are associated in tilapia farming systems to regulate the density of the tilapia population in view of the high tilapia prolificacy.

However, the expansion of catfish farming in Africa appears to be very slow on a global scale; Its reproductive cycle seems too long and not yet mastered by fish farmers.

In order to overcome the difficulty of obtaining the juveniles of African catfish in ponds, agronomic research (aquaculture) has developed a technique of rapid and assisted multiplication of catfish from African catfish. This technique has been successfully tested at the Gimbi Station in the DRC.

Our communication aims to share with the community gathered at the World Aquaculture Conference 2017 in Cap-Town, the rapid and assisted multiplication technique of african catfish as experienced at the Gimbi Station in the Democratic Republic of Congo.

This simple technique we recommend is to stimulate the maturation of ovocytes in female catfish, harvest ovocytes, extract spermatozoa from male catfish, fertilize ovocytes and harvest larvae after hours, Incubation with very encouraging results in terms of availability of African catfish juveniles.



From left to right, a female parent, a male and fertilized eggs incubated in a water basin.  
Picture of Joachim Kuzimbila, DRC

## THE IMPACT OF TEMPERATURE ON THE REGULATION OF INTERFERON REGULATORY FACTOR 4 (IRF-4) IN NERVOUS NECROSIS VIRUS-INFECTED ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

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Global climate change and extreme weather both event influencing marine environment causing sea temperature unstable attributing the impact on aquaculture productivity and incidence of aquatic diseases outbreak where relatively warmer sea temperature promotes virus proliferation and transmission. One of the aquatic virus, nervous necrosis virus (NNV) belonging to family Nodaviridae has been previously demonstrated the effect of temperature on the ability of NNV infection was assessed as a possible evidence for seasonal variation attributed to the incidence of viral nervous necrosis disease (VNN). During virus infection, interferon (IFN) immune responses are considered as the early host defense mechanism and the expression of IFN is cooperative initiate and regulates by a transcription factors of the interferon regulatory factor (IRF) family. Among, IRF-4 has been reported as a negative transcription factor in immune response as well as a vital transcription partner regulates the thermogenic expression to maintain cold tolerance. Hence, IRF-4 may be an important factor correlates between thermal stress regulation and viral-induce immune response.

In this study, IRF-4 in orange-spotted grouper was study the effect of temperature on the expression pattern of IRF-4 in NNV-infected orange-spotted grouper. The constitutive high expression of *osgIRF4* was detected in the gill, head kidney, spleen, trunk kidney, and thymus. The transcript of IRF-4 was responsive to viral mimic poly I:C stimulation and imply that it may play an crucial role in viral induced-immune responses. When fish larvae were exposed to low (20°C) and high (36°C) temperature, the expression of *osgIRF4* elevated drastically which support its thermogenic role. When NNV-infected GF-1 cells exposed to low temperature, *osgIRF4* was induced at 24h, whereas high temperature up-regulate *osgIRF4* expression at 12h which earlier than low temperature. Hence, IRF-4 may be one of a key thermogenic regulator involved in NNV infection during winter and summer.

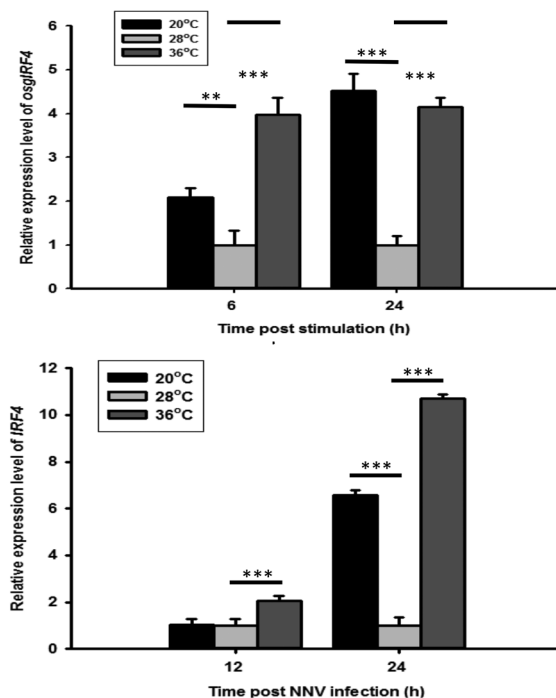


FIGURE 2. Effects of temperature changes on NNV infection in GF-1 cells. Relative IRF4 levels in GF-1 cells at 12h and 24h after virus infection was measured by real-time PCR and normalized to  $\beta$ -actin.

## NATURAL ENVIRONMENTAL VARIATION: 'FRIEND OR FOE' FOR SCOTTISH BLUE MUSSEL *Mytilus edulis* CULTIVATION

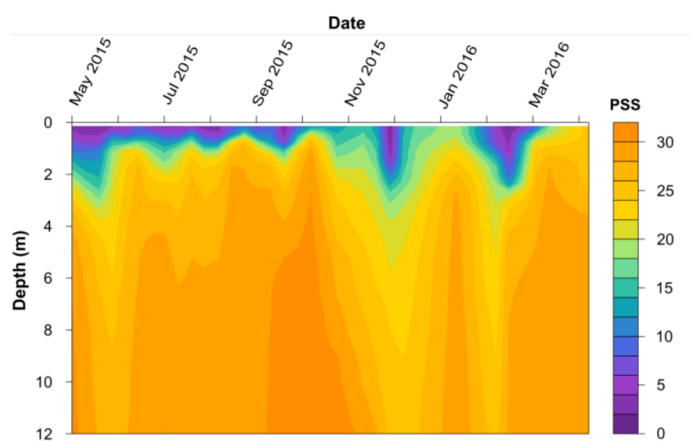
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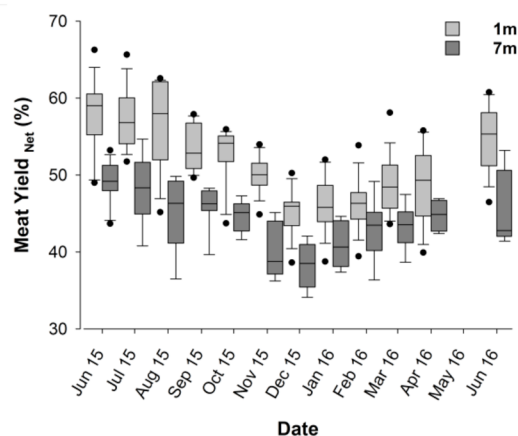
Populations of blue mussels *Mytilus edulis* thrive in complex habitats, naturally exposed to environmental variation on both large and small spatial and temporal scales. Considering the economic importance of mollusc aquaculture, relatively little is known of culture optimization of mussels grown in the wild. Here we determine mussel quality under typical longline cultivation exposed to large variations in seawater environmental conditions.

We monitored fluctuations in key environmental drivers (temperature, salinity, food availability, seawater carbonate chemistry) and the corresponding quality of the cultured mussels (condition index, meat yield) over one year at a mussel farm on the west coast of Scotland, UK. Whilst both the environment and mussel product quality varied seasonally as expected, the most dramatic responses were with depth i.e. down the length of a mussel cultivation rope. The range in water salinity of near surface grown mussels varied from 1.8 to 29.2 PSS within days, with far less variation for deeper grown mussels ( $27.0 \pm 3.7$  PSS at 7m) (Fig 1). Seasonal and vertical fluctuations in temperature, salinity and food availability, in particular were closely associated with mussel quality. Meat yield and condition index were highest in summer and decreased towards winter. Maximum meat yields were reached in near-surface grown mussels (51 % at 1m) and gradually decreased with depth (44 % at 7m) (Fig 2)..

We have shown that a thorough characterisation of the water column, over at least one year, is necessary to optimise site selection in mussel cultivation. Here it is suggested that warmer water temperatures and higher food availability favour mussel growth and quality in near surface cultivation. Such data may provide management guidance for the industry, such as the evaluation of improved production habitats and suitable product harvest times, including the placement of cultivation ropes.



**Fig 1:** Variation in water salinity at a mussel farm (Lat: 56.710, Lon: -5.029) over time and depth (CTD data).



**Fig 2:** Annual variation in *Mytilus* meat yield from 1m (light grey) and 7m (dark grey) depth.

## THE OCCURRENCE OF *Fusarium* SPECIES AND THEIR MYCOTOXINS IN SOUTH AFRICAN ABALONE FEED

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Abalone (*Haliotis midae*) aquaculture is the most developed and successful sector of the South African aquaculture industry. A productive growth rate is dependent on a steady supply of high quality feed. Currently most farms make use of formulated feed, which is processed from locally sourced grains and animal protein. Contamination of feed with filamentous fungi and their associated mycotoxins is therefore a predictable consequence. Infection of grains with mycotoxigenic fungi species may occur from the early stages of seed set until harvest. Most *Fusarium* species are able to produce one or more mycotoxins with varying degrees of toxicity. Fumonisin, trichothecenes and zearalenone are the major mycotoxin groups associated with *Fusarium* spp. Mycotoxins are toxic to humans, terrestrial- and aquatic animals. Mycotoxins are structurally very diverse, a characteristic that leads to a wide range of symptoms. Zearalenone and trichothecenes are both heat resistant and water insoluble. In contrast fumonisins are water soluble and can bind to proteins or other matrix components during feed processing. The aims of this study, were to determine the occurrence of mycotoxigenic *Fusarium* spp. and their associated mycotoxins abalone feed. Secondly to determine the effect of the aquatic environment on fumonisin contaminated abalone feed.

Abalone feed and raw materials were screened for *Fusarium* spp. and related mycotoxins. Standard morphology were performed in parallel with multigene molecular species identifications. Furthermore, sterile feed were inoculated with *F. verticillioides* and incubated. Following, fumonisin contaminated feed were submerged in sterile seawater for 24 hours. Mycotoxin analyses were performed on both the feed and water.

The two most dominant *Fusarium* spp. that were isolated, include *F. verticillioides* and *F. subglutinans*. Fumonisin B<sub>1</sub> and B<sub>2</sub> were detected in raw materials used in the formulation of abalone feed. Detected levels were substantially lower than the recommended allowance for materials used in catfish feed according to the FDA. Mycotoxin risk assessments to establish the maximum tolerable levels of allowable fumonisin contamination in abalone feed have not been reported yet. The study furthermore showed that that fumonisins leach out of the feed into the seawater.

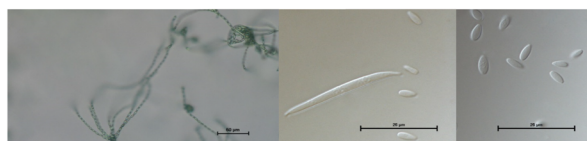


Figure 1: *Fusarium verticillioides* morphology. Left to right, microconidia *in situ*, macroconidia, microconidia.

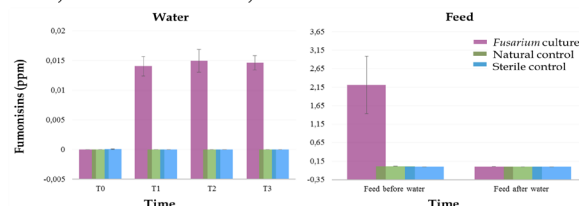


Figure 2: Total fumonisin concentrations, Left to right, in water at time intervals and in abalone feed measured before and after submerged in water.



## EVALUATING PARTICLE PACKAGING AND TRANSPORT AT AN ACTIVE AREA OF SALMON AQUACULTURE OVER A MIXED GRAIN SIZE SEABED

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As part of the Ecosystem Response to Aquaculture (ERA) project at the Ratteran and Ornoya salmon aquaculture farm sites in Froya, Norway, instrument pods were deployed in August 2015 and again in May 2016. The instrument pods (Fig 1 A and B) included:

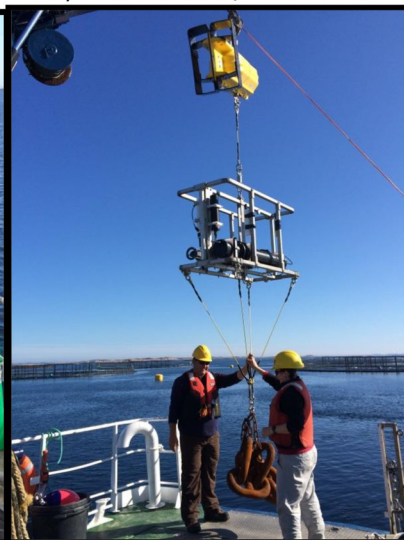
- A) A size versus settling camera inside a stainless steel pressure housing with attached settling column which measured settling particles from 200µm to mm size scale and sat just above the seabed. In addition the instrument pod contained a Nortek high resolution Aquadopp current meter which measured near bed currents and waves, an RBR wave gauge and RBR turbidity sensor.
- B) A second instrument pod that was moored 1m off the seabed contained a Machine Vision Floc Camera (MVFC) and LISST 100x which measured particle size in-situ from 1µm to mm size. In addition the pod contained an RBR CTD measuring salinity, temperature and pressure along with dissolved oxygen and turbidity. An eco-BB instrument from Wetlabs measured Coloured Dissolved Organic Matter (CDOM) and Chlorophyll concentration as well as backscattering in the red which gives an idea if particles are organic or inorganic in nature.

The data will be discussed in terms of the particle size, packaging and transport of possible aquaculture derived waste material from the farm site. These data will help explore linkages between organic matter transport, its deposition and possible effects in the near field and far field associated with aquaculture operations and understanding salmon aquaculture sustainability in the marine environment.

A) Settling Pod w/ Aquadopp



B) Floc Camera / LISST Pod



## CONFLICT BETWEEN SOMATIC AND GONADAL GROWTH IN SEA URCHINS: IMPLICATIONS FOR AQUACULTURE

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Studies on aquaculture of sea urchins have focused on production of large, high quality gonads by large individuals. Sea urchins must reach a threshold of body size before they are marketable. Decreasing the length of time required to reach this threshold is desirable for aquaculture of sea urchins. Somatic growth in sea urchins is great initially and then decreases with size as gonadal production occurs. Somatic growth in *Strongylocentrotus intermedius* is initially fast, then decreases after initial HD = ca. 15 mm with increasing size to an asymptote (Fig.1). Gonads of *S. intermedius* begin to increase greatly in size after a HD = ca. 15 mm (Fig. 2). The marketable size of *S. intermedius* is ca. 50 mm.

Gonadal production exceeds somatic production seasonally in species with annual reproductive cycles, suggesting conflict. Conflict between somatic and gonadal production requires limited common nutrient requirements. Independent processes would suggest differences in nutrient requirements or preferential allocation. Scheibling and Hatcher (2013) concluded there is apparent conflict between somatic and gonadal growth in strongylocentrotids but that the hypothesis that they are independent could not be rejected. Field and laboratory studies have shown both somatic and gonadal production are related to food availability and quality. Species with a low allocation to somatic production have faster growth and greater gonadal production, indicating a genetic basis for production. Intrinsic control (hormonal) of allocation to gonadal production but not to somatic growth has been reported. Rapid growth to a marketable size is important in aquaculture. The studies suggest feeding a high quality feed would increase the rate of somatic growth and decrease time to marketable size but the increase in gonadal growth would decrease cost effectiveness.

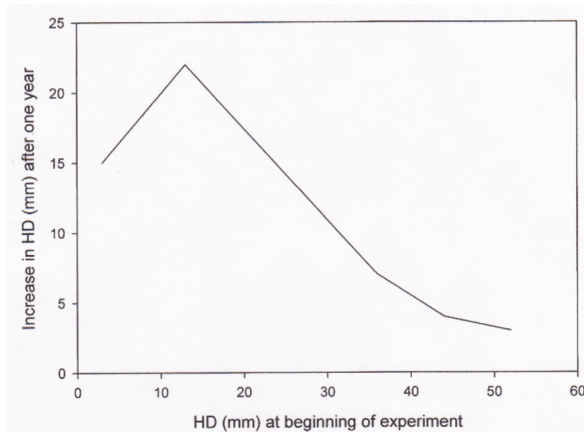


Fig. 1. Somatic growth curve of *Strongylocentrotus intermedius*. From data in Fuji, 1967.

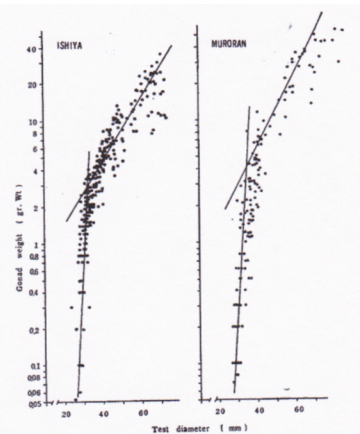


Fig. 2. Gonadal production and body size in *S. intermedius*. From Fuji, 1967.



# GROWTH PERFORMANCE OF THE VERY SMALL ROTIFER *Proales similis* IS MORE DEPENDENT ON LIVE LACTIC ACID BACTERIA AND BACILLUS SUBTILIS THAN THE BIGGER ROTIFER *Brachionus rotundiformis*

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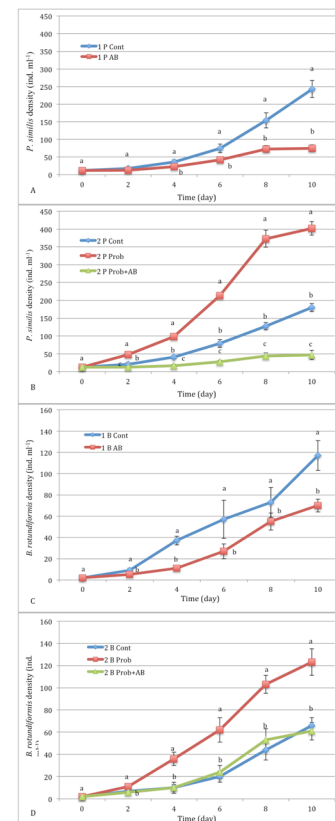
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*Proales similis* and *Brachionus rotundiformis* are commercially cultured species, which predominantly feed on microalgae. However, the importance of the bacterial community composition on growth performance of *P. similis* and *B. rotundiformis* culture is still unknown. In this study, the effect of limiting the bacterial growth and, as a second aim, the effect of the addition of a small amount of live or dead bacteria on the growth performance and microbial community (MC) of *P. similis* or *B. rotundiformis* culture was evaluated for a 10 day culture period. A rotifer culture with non-manipulated MC and fed autoclaved algae was used as the first control, and a culture started in autoclaved sea water and fed autoclaved algae was used as the second control. In order to test a feed effect, probiotics and other bacteria present in the culture system were inhibited to grow through the addition of an antibiotic mixture (AB) and the rotifer culture performance was compared to that of a culture to which live probiotics were added.

In the presence of the live probiotic mixture, both rotifers species showed a better growth performance than those without the presence of probiotic mixture or those with the AB added. In addition, the growth performance of the rotifer *P. similis* is more dependent on live lactic acid bacteria and *B. subtilis* than the rotifer *B. rotundiformis*. (Figure 1)

The supplementation of these probiotic bacteria not only increased the production of the rotifers, but also had a regulating effect on the microbiota. At day 6 and 10, the bands from the *L. plantarum*, *B. subtilis*, *L. brevis*, *P. acidilactici*, *P. pentosaceus* and *L. fermentum* were dominant in the treatments started in autoclaved water, fed autoclaved algae with the addition of the mixture of six species of tentative live or killed probiotic bacteria.

Figure 1: Growth performance of *P. similis* (A and B); *B. rotundiformis* (C and D) under five different culture conditions.



## ROLE OF SALINITY ON GROWTH PERFORMANCE OF *Oreochromis niloticus* AND *Oreochromis urolepis urolepis* HYBRIDS

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Influence of salinity on hybrids descended from *O. niloticus* and *O. urolepis urolepis* was investigated for 63 days. A total of 120 fry of  $0.29 \pm 0.01$ g were stocked in 1m<sup>3</sup> plastic tanks at a density of 10 fish / m<sup>3</sup> per tank. The experiment involved three salinity treatments 15, 25 and 35 with fresh water (2 salinity units) as control. The hybrids were fed on a balanced diet of 40% crude protein at 5% body weight twice a day. Water quality parameters were measured once a week. However, results on SGR, average weight gain and survival rate were not significantly affected by salinity ( $p > 0.05$ ). FCR differed significantly among treatments ( $p < 0.05$ ). The 25 Practical Salinity Unit (PSU) showed better growth performance than other treatments. Surprisingly all treatments showed better growth than control. When examined at the end of the study all hybrids were found to be 100% males. Length-weight relationship “b” values and condition factor “K” showed that hybrids had isometric growth, good health and were properly managed. It was concluded that if proper management is followed, the studied hybrids can be good candidates in both intensive and semi intensive mariculture by coastal people. The hybrids can solve the problem of stunted growth, be an alternative to the use of hormones in sex reversal.

## THE PINEAL COMPLEX OF THE RIVER LAMPREY *Lampetra fluviatilis* (L) AS A PHOTSENSORY ORGAN – A LIGHT AND ELECTRON MICROSCOPY STUDY

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The organization of the pineal complex in lampreys has been studied in few species. Differences in lifestyle (migratory vs. settled), nutrition and environmental conditions among lampreys point to the need of detailed comparative investigations. The present study was performed to characterize the pineal complex in the river lamprey *Lampetra fluviatilis* (L). The adult lampreys were taken up from the Vistula Lagoon (Baltic Sea, Poland) and anesthetized with 2-phenoxyethanol. Immediately after decapitation, the head part containing the pineal complex was dissected and processed for light and electron microscopy.

The pineal complex of the river lamprey was located beneath a specialized part of the integument, which was highly translucent and visible as a white-yellow spot on the dorsal surface of the head. The skin covering the pineal region was completely devoided of pigment cells and the epithelium was markedly thinner than in the neighbouring area. Under the skin, the specific connective tissue formed a truncated cone-like structure with a base directed outside and an apex directed to the pineal complex. It created a deep invagination around the pineal complex. The connective tissue fibres, separated by amorphous substance, ran perpendicular to the head surface and focused on the pineal complex. From morphological point of view, this structure may be considered as “a condenser formed by a bundle of optical fibres”. The pineal complex comprised the pineal organ and the parapineal organ. The long axis of the pineal complex was directed dorso-anterior that may help to recognize the direction of light source. The pineal organ was formed by an oval-shaped end-bulb with large lumen, an atrium and a solid pineal stalk. The wall of the end-bulb resembled the stratified epithelium and was more thicker in the ventral than in the dorsal part of the bulb. It was formed by photoreceptor cells, supporting cells and neurons. The ultrastructure of these cells differed markedly between ventral and dorsal parts of the end-bulb wall. In the ventral part, photoreceptor cells had the well-developed outer segments, variable in shape, containing from 30 to 120 discs as well as the inner segments with numerous mitochondria. Immunohistochemical studies demonstrated the presence of rhodopsin and iodopsins in the apical protrusions of these cells. The upper parts of supporting cells comprised numerous guanine-like crystals. These light-reflecting crystals probably act as a mirror and increase illumination of the photoreceptive segments. In the dorsal part of the end bulb, many photoreceptors cells showed the presence of oval apical-protrusions instead of outer segments. The differences in organization of apical parts of photoreceptor cells are probably related to the presence of various photopigments. Summing up, according to the morphological criteria the pineal complex of *Lampetra fluviatilis* is highly specialized photosensory organ that is able to provide a precise information about the intensity, color and direction of light.

Supported by National Science Center in Poland (grant No. DEC-2013/09/B/NZ9/03130) and KNOW (Leading National Research Centre) Scientific Consortium “Healthy Animal - Safe Food” (decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015).

EFFECTS OF TWO COMMERCIAL DUSKY KOB (*Argyrosomus japonicus*) DIETS AND A TRADITIONAL TROUT MEAL ON GROWTH OF JUVENILE DUSKY KOB

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Due to the high cost of fish feed within the finfish aquaculture industry, the need to develop cost effective feed which will maximise growth exist. This study therefore, compared the effects of different diets (2 commercial dusky kob diets and a traditional trout meal diet) on the growth of juvenile dusky kob (*Argyrosomus japonicus*). The three diets had an estimated crude protein content of 50%. Fifty juvenile dusky kob (mean body mass  $28.18 \pm 2.19$ g, sourced from a commercial fish farm) per tank were distributed across 18 recirculating polyethylene tanks which were divided into six replicates per diet. Fish were fed 2.8% of its average body mass daily, followed by weekly measurements of length and weighed for six weeks. Trout meal produced a marginally superior mass gain compared to the two commercial dusky kob diets (Fig.1). The statistical analysis showed that after 6 weeks of feeding, the effect of the three feed treatments on both fish length and mass was significantly different ( $P < 0.05$ ). The feed conversion ratio (FCR) between the three diets was not significantly different. The FCR values for the three diets (kob diet 1, kob diet 2 and trout meal) were 5.76, 5.33 and 5.37. During feeding it was observed that the pellets in kob diet 1 were floating at the surface which led to a quick deterioration in water quality and possible nutrient leeching, while pellets of kob diet 2 was denser and sank to the tank’s bottom immediately after coming into contact with the water surface. The pellets of the trout meal stayed mostly in the middle water column which allowed enough time for the fish to feed, as the majority of experimental fish preferred to feed in the middle of the water column. It is therefore recommended, that extrusion methods be adjusted during the production of pellets for the kob diets, in order to obtain same buoyancy levels as that found in the trout meal, but without compromising the nutritional composition of the pellets.

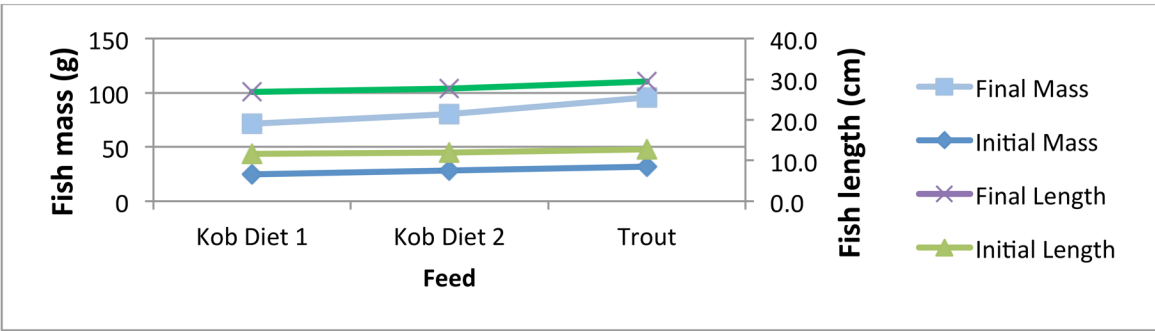


Figure 1. Variations in length and mass gain in response to the three diets. Data point represents the means of six replicates  $\pm$  standard error.

# INTEGRATED MULTI-TROPHIC ECOLOGICAL AQUACULTURE TECHNOLOGY FOR SHRIMP POND AQUACULTURE IN CHINA

Jian Li\*, Ping Chen, Ping Liu, Zhiqiang Chang and Jitao Li

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China's marine shrimp and crab aquaculture yield reached 1.435 million tons in 2015, and was accounting for 61.0% of China marine aquaculture production, mainly including shrimp (*Litopenaeus vannamei*, *Penaeus monodon*, *Fenneropenaeus chinensis* and *Marsupenaeus japonicas*) and crab (*Portunus trituberculatus* and *Scylla serrate*). However, there are several problems in the current shrimp farming, such as lower feed utilization rate, weak disease prevention capacity, poor technology targeted. Yellow Sea Fisheries Research Institute (YSFRI) set up the integrated multi-trophic ecological shrimp aquaculture model in ponds. This shrimp aquaculture model includes a series of key technologies, such as biological prevention and control, micro-porous aeration, usage of living creature diets and immune enhancement, and has achieved great successes in the north of China.

## Establishment of shrimp IMTA in pond

Species of the shrimp IMTA in pond includes Chinese shrimp, Swimming crab, shellfish and Fish. In Spring, the pond sediment is tonhaltig or pink silty clay, and then set up the protective net covering clams, and crab foster pond.

## Application of bottom micro-pore aeration

It's oxygen-poor at the bottom of pond, Dissolved oxygen (DO) content at the bottom is lower than 2 mg/L. Micro-pore aeration is less resistance, small bubbles, complete dissolved oxygen, and OD values in the middle of pond bottom is 4.5. DO values of micro-pore aeration between the upper and lower layer were less than 0.1 mg/L. The changes of different water layers of stone aeration are significant (0.21-0.58 mg/L). Micro-pore aeration makes DO distribution more uniform.

## Usage of living creature diets

To improve the nutrition and survival of the shrimp, rotifers and artemia were used as strengthen bait during the shrimp larvae period. From Zoea 2, artificial diet group reduce gradually to 61.68% at postlarvae, while the other groups remained about 80%. Live bait, such as *Ampithoe valida* could significantly improve the growth rate and nonspecific immunity of Chinese shrimp larvae.

Table 1 The economic performance of shrimp IMTA in pond

| Species                 | Quantity (per mu) | Size (per kg) | production (kg/mu) | Survival rate (%) | Output (yuan/mu) |
|-------------------------|-------------------|---------------|--------------------|-------------------|------------------|
| Chinese shrimp          | 6000              | 25-35         | 61                 | 33.2              | 5490             |
| Shellfish               | 50000<br>~60000   | 120-160       | 280-380            | 78.0              | 1980             |
| Swimming crab           | 2000<br>~3000     | 4-7           | 28.5               | 10.32             | 1282             |
|                         |                   | 3.5-5.5       | 34.5               |                   | 3372             |
| Half-smooth tongue-sole | 20                | 870 g         | 14.8               | 85                | 2960             |

## Immune enhancement approach

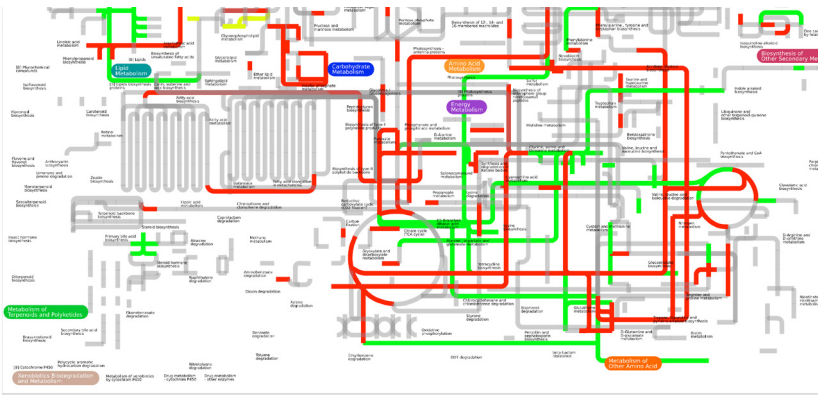
Several immunostimulants were used in the pond IMTA successfully, such as anti-stress vitamins (Vc, Ve), Chinese herbs, alga extracts and probiotics. The results found that the immunostimulants could improve the immunology and raise disease resistance of the shrimp.

METABOLICS AS A FUNCTIONAL TOOL IN COMBATING ANTIBIOTIC-RESISTANT VIBRIO ALGINOLYTICUS

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To develop a low-cost and “green” approach to control infection caused by *Vibrio alginolyticus*, GC-MS based metabolomics approach was used to investigate metabolic profiles of survival and dead *Danio rerio* post the infection with antibiotic-resistant *V. alginolyticus*, and identify key pathways and crucial metabolites that were related to the survival and death. Compared with the control without bacterial infection, thirty-six and thirty-seven metabolites were differential at  $P < 0.05$  in survival and dead groups, respectively. Two were higher and thirty-five were lower in the death group, while sixteen were increased and twenty were decreased in the survival group. Among the enriched pathways, a reversal metabolic status on the TCA cycle forms a characteristic feature between the death-related metabolome and the survival-related metabolome, being reduced and elevated TCA cycle in the death-related metabolome and the survival-related metabolome, respectively. Exogenous malate promote *Danio rerio* survival in a dose-dependent manner. Correspondingly, activity of pyruvate dehydrogenase (PDH),  $\alpha$ -ketoglutaric dehydrogenase (KGDH) and succinate dehydrogenase (SDH) were decreased in the death group and increased in the survival group. Exogenous malic acid elevated activity of KGDH and SDH. These results indicate that the TCA cycle is a key pathway responsible for survival or death in response to infection caused by *V. alginolyticus*, and highlight the way on development of a low-cost and “green” approach to control infection through metabolic modulation. The approach has been demonstrated in *V. alginolyticus* with different antibiotic-resistant mechanisms.

Fig 1 Metabolic network pathways in *Danio rerio* challenged with *V. alginolyticus*. Red, increase in survival group and decrease in death group; blue, increase in death group and decrease in survival group; yellow, increase in both death group and survival group; green, decrease in both death group and survival group.



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| Total                   |                      |                  |                       |                      | 15084               |



## THE BIOLOGICAL AND TOXICOLOGICAL EFFECTS OF OXYTETRACYCLINE AND SULFAMETHOXAZOLE AT LEVELS USED IN AQUACULTURE AND THEIR ENVIRONMENTAL CONCENTRATIONS IN NILE TILAPIA *Oreochromis niloticus*

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Antibiotics are used globally to treat/prevent human and animal diseases in addition to their application as growth promoters for livestock animals and aquatic species. They exist ubiquitously in the environment because of misuse, overdose and poor absorption after ingestion coupled with their high-water solubility and degradation resistance. However, it is not known whether antibiotics used in aquaculture and their environmental concentrations exert similar biological and toxicological effects in fish. This study explored the biological and toxicological effects of oxytetracycline (OTC) and sulphamethoxazole (SMZ) in diets and bath treatments in Nile tilapia, *Oreochromis niloticus*.

Twenty *O. niloticus* were divided into five groups. The first two groups were subjected to a feed containing OTC and SMZ for 84 days. The other two groups were exposed to water (bath treatment) containing similar types of antibiotics based on their environmental existence for the same period. The last group was not treated with antibiotics to represent the control.

Results indicated that, both antibiotics significantly reduced growth performance by impairing feed efficiency, digestive enzymes activities, nutrient digestibility and whole body composition in treated *O. niloticus* than control. Similarly, both antibiotics caused hepatotoxicity, lipid peroxidation and induced oxidative stress by affecting antioxidant capacity in the treated than control *O. niloticus*. Furthermore, they stimulated inflammatory response, affected fish immunity and induced genotoxicity in treated *O. niloticus* compared to the control group.

Taken together, it is clear that, exposure to aquaculture and environmental antibiotics cause relatively similar biological and toxicological effects in *O. niloticus*. Future studies should explore the possible potential risks resulting from consuming antibiotic contaminated fish in both environments.

## YEAST CULTURE DIETARY SUPPLEMENTATION MODULATED GUT MICROBIOTA AND IMPROVED GROWTH OF GRASS CARP *Ctenopharyngodon idella*

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Gut microbiota contributes positively to the physiology of their host. In animals, prebiotics including yeast culture (YC), were widely used to improve host health and stimulate growth performance by modulating gut bacteria species. In the present study, we used high-throughput sequencing of 16S rRNA gene to assess the effects of YC dietary supplementation on the gut microbiota and their metabolic capacity in grass carp. The results revealed an evident change on compositions and relative abundance of gut microbiota by different YC-dietary treatments. PCoA test showed that gut bacterial communities in the control and Treat3 formed distinctly separate clusters, while no significant separations were found in other treatments. Treat3 also showed a significant increase of the proportion of phyla Proteobacteria and Actinobacteria, particularly the genera *Stenotrophomonas*, *Pseudomonas*, *Phyllobacterium* and *Rhodococcus*, as compared to control. Compared with other treatment groups, Treat3 contained the highest proportion of the putative probiotics and obviously enhanced the amino acid, lipid metabolic capacity and digestive system of the gut microbiota. Moreover, after ten weeks feeding, the Treat3 and Treat4 showed good growth performance and biochemical parameters. These results indicated that certain YC dietary treatment modified the composition and the metabolic capacity of gut microbiota in grass carp, as well as the growth performance.

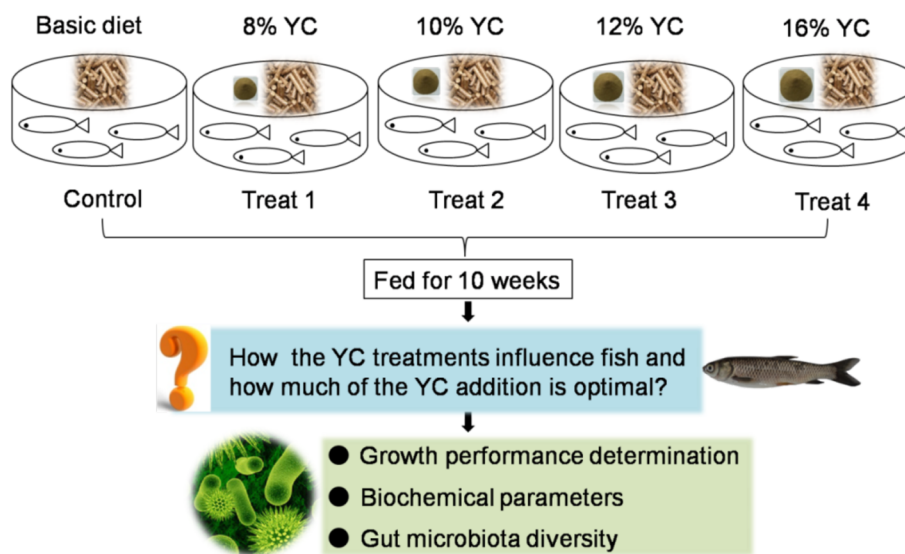


Figure1. Schematic representation of experimental design

## GENOMICS AND ITS APPLICATIONS IN AQUACULTURE

Zhanjiang (John) Liu

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Great advances have been made in aquaculture genomics including generation of the reference genome sequences, high density and high resolution genetics maps, analysis of genomic diversity and variations, development of efficient genotyping array technologies, and application of genomics for analysis of performance and production traits through quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS). In this presentation, I will use catfish as an example to summarize research progress made in aquaculture genomics, whole genome sequencing, improvements of whole genome sequence assembly, scaffolding, and construction of chromosome-level sequence assemblies. With the reference genome sequences, the relationship of whole genome variations and performance or production traits can be dissected using RNA-Seq, bulk segregant RNA-Seq (BSR-Seq), QTL mapping or GWAS. We have devoted much effort towards understanding important performance and production traits including disease resistance, growth, processing yields, low oxygen tolerance, heat tolerance, and body conformation using genomic approaches. Earlier research focused on a large set of known genes using microarrays, whereas our most recent studies have adopted the next generation sequencing approaches such as RNA-Seq, BSR-Seq, and GWAS for the analysis of candidate genes involved in performance and production traits. Harnessing genomic techniques, especially GWAS work using the high density 690K SNP arrays, it is now not only feasible, but also practical to utilize genomics and genome selection for aquaculture. Detailed data and processes of using genomics for aquaculture issues will be presented. The genomics revolution is here for aquaculture. The potential is huge, and the opportunities are great. Some challenges will also be presented and potential solutions will be discussed.

## SELECTIVE BREEDING OF SWIMMING CRAB *Portunus trituberculatus* IN CHINA

Ping Liu <sup>a,b,\*</sup>, Jian Li <sup>a,b</sup>, Baoquan Gao <sup>a,b</sup>, Jianjian Lv <sup>a,b</sup>, Xianliang Meng <sup>a,b</sup>

<sup>a</sup> Key Laboratory of Sustainable Development of Marine Fisheries, Ministry of Agriculture, P.R.China, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 266071 Qingdao, China

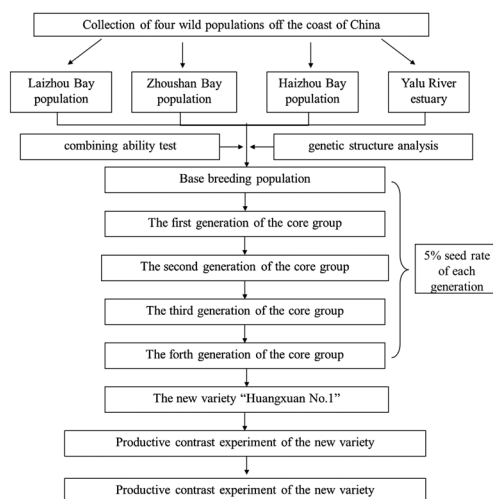
<sup>b</sup> Laboratory for Marine Fisheries and Aquaculture, Qingdao National Laboratory for Marine Science and Technology, No. 1 Wenhai Road, Aoshanwei Town, Jimo, Qingdao, China

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The swimming crab, *Portunus trituberculatus* (Crustacea: Decapoda: Brachyura) is widely distributed in the coastal waters of Asia-Pacific countries. The farming of *P. trituberculatus* has been developing rapidly in China over the last decades and the highest yield of 100,000 tons with 40,000-ha farmed area. However, the swimming crab harvests have declined drastically with frequent outbreaks of disease. Moreover, the wild fishery ground and fishery season of this crab are almost disappeared in many regions since 1990s. In order to enrich this resource, scientists at the Yellow Sea Fisheries Research Institute (YSFRI) worked to select and breed a new variety of *P. trituberculatus* with improved growth performance since 2005. In 2010, they have produced a fast-growing population called “Huangxuan No.1” after five generations of selection. Further new species selective-breeding research is ongoing.

The goal of the breeding program is to develop a faster-growing cultured crab (*P. trituberculatus*) through population selection strategy. In 2005, a mating design is applied. The base selective population was established after finishing the combining ability test and genetic structure analysis. Each generation selective intensity was around 5% to select the bigger ones. After mating, the females migrate to the pond indoor for overwintering. Females extrude a brood of fertilized eggs with stored sperm, then moved to the cultivate pond indoors. The released larvae, termed zoea, are advected to indoor farming pond where they go through four zoea-stage larvae, one megalopa and juvenile stage. At juvenile II stage stage, randomly sampled animals were moved to the ponds outdoor separately. The ponds were separated into several parts with net.

Till date, the new variety “Huangxuan No. 1” for fast-growing of *P. trituberculatus* was selected successfully in 2010 after five generation selection from four geographical population, and the body weight increased 20.12% and survival rate increased 32.00%, respectively, compared with the unselected population.



## MOLECULAR CHARACTERIZATION OF THIOREDOXIN-LIKE PROTEIN 1 (ShTXNL1) FROM BIG-BELLY SEAHORSE *Hippocampus abdominalis* WITH ITS IMMUNE RESPONSES

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Thioredoxin is a highly-conserved protein, can be found in both prokaryotes and eukaryotes. Reactive oxygen species (ROS) are produced in response to general metabolic processes, radiation, metal oxidation and pathological infections. High levels of ROS lead to cell death via autophagy. However, thioredoxin acts as an active regulatory enzyme under excessive levels of ROS. Here we have performed *in silico* analysis to check the structural properties of seahorse thioredoxin-like protein 1 (ShTXNL1). Evolutionary identification showed ShTXNL1 protein belongs to the thioredoxin superfamily. ShTXNL1 possesses an active thioredoxin domain consisted of 289 amino acids with C-terminal proteasome-interacting domain (PITH), a component in 26S proteasome which helps to bind to the matrix or cell from the C-terminal site. Pairwise alignment results proved that sequence matched to *Hippocampus* species with 99.0 % sequence identity and 99.7 % similarity. Conserved thiol-disulfide Cysteine residue containing Cys-X-X-Cys motif can be found in the first few amino acids in the second beta sheet starting from N-terminal. This motif can be discovered in fish species as <sup>14</sup>CRPC<sup>17</sup> in the teleost sequence while in mammals it can found as <sup>14</sup>CGPC<sup>17</sup>. ShTXNL1 consisted with two N-linked glycosylation sites at <sup>72</sup>NISA<sup>75</sup> and <sup>139</sup>NESD<sup>142</sup>.

Quantitative real time PCR indicated, the highest expression was in muscle followed by ovary, brain, gill and blood tissue among fourteen different tissues from healthy seahorses. As these tissues required a high amount of energy, generation of ROS may relatively high in those tissues. Thus, in order to scavenge the ROS intensive expression of ShTXNL1 can be observed in muscle. Gill and the blood tissues were subjected to the immune challenge with Lipopolysaccharides, *Edwardsiella tarda* and *Streptococcus iniae* to observe the modulation of the transcription pattern of ShTXNL1. According to the results, ShTXNL1 showed an immediate response (at 3 h and 6 h post infection) in gill towards bacterial stimuli. Oxidative burst and the membrane damage that leads by lipid peroxidation can be occurred with the invasion of a bacterial pathogen. Up-regulation of ShTXNL1 can be observed at 3 h, 24 h and 72 h post infection in blood tissue against the bacterial stimuli. Altogether, the ShTXNL1 gene can be identified as an immunologically important gene in seahorse.

## TOWARD DETERMINING THE DIETARY LYSINE REQUIREMENT IN THE SOUTH AFRICAN ABALONE *Haliotis midae*

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Department of Ichthyology and Fisheries Sciences  
Rhodes University

Animals generally do not have a requirement for protein, but instead have a requirement for specific essential amino acids (EAAs) and non-essential amino acids (NEAAs). The NEAAs are those that can be synthesised by the animal, however, EAAs cannot be synthesised and must therefore be supplied as part of the diet. When these amino acids (AAs) are supplied in the correct ratios and with the correct level of digestible energy, nutritionists can maximise somatic growth from proteins. Crystalline AAs have been used as a tool in feed formulation research in order to quantify the AA requirements in aquaculture species. In common with other water soluble nutrients, leaching of crystalline AAs from diets prior to ingestion is of concern in an aquatic environment. Microencapsulation techniques have been successfully employed to restrict micronutrient leaching, and improve ingestion rates. In this research, LysiPEARL™ (Kemin®) was used as a means to determine the lysine requirement in *Haliotis midae*.

It has been proposed that crystalline AAs are not suitable for AA studies in *H. midae* due to the slow feeding rates of the species as well as the solubility of these AAs. However, 90.00 % of supplemented lysine was maintained in this study after a six hour period of leaching. Six isoeNERgetic (15.90 MJ/kg), isolipidic (6.00 %) and isonitrogenous (29.00 %) diets enriched with 5.52, 6.40, 7.28, 8.14, 9.00 and 9.86 % lysine (as a % of protein) were fed to triplicate groups of 20 *H. midae* (20.41 ± 1.95 mm SL 1.51 ± 0.44 g w.wt) for 90 days. Wet weight and shell length measurements were taken every 30 days and various growth indices (SGR % body weight.day<sup>-1</sup>, FCR, PER, feed consumption % body weight.day<sup>-1</sup> and condition factor) were calculated.

Linear regression showed that FCR increased as dietary lysine increased (Regression analysis,  $p=0.031$ ), and that PER reduced as dietary lysine increased (Regression analysis,  $p=0.026$ ). Feed consumption also increased as dietary lysine increased (Regression analysis,  $p<0.001$ ). The inclusion of lysine at 7.28 % of the total protein in the diet resulted in significantly superior SGR (0.57±0.01 % body weight.day<sup>-1</sup>) to that of 5.52 % (0.42±0.05 % body weight.day<sup>-1</sup>), FCR (1.51±0.05) to that of 8.14 % (1.99±0.21) and PER (2.45±0.07) to that of 8.14 % (1.99±0.18; ANOVA,  $p<0.05$ ). There was a significant difference found in feed consumption (% body wt.d<sup>-1</sup>), with consumption increasing significantly between the first three dietary treatments and the last three dietary treatments (ANOVA,  $p<0.001$ ). There was no significant improvement in SGR when dietary lysine increased above 7.28 % of the dietary protein in the diet. The diet producing the best SGR, PER and FCR in this study was diet 3 which had a measured lysine content of 6.90 %. The results of the present study suggest that the lysine requirement in *H. midae* is in the range of 6.00 - 7.00 % of dietary protein. From these data amino acid ratios were used to estimate optimum inclusion levels of other essential amino acids. However, lysine availability in LysiPEARL™ may have resulted in over estimations due to the lipid encapsulation technique used, and haliotids limited ability to efficiently digest lipids. For this reason EAA requirements were suggested based on three different hypothetical scenarios of lysine availability from LysiPEARL™.

## PACIFIC OYSTER COCULTURE GROWOUT ASSESSMENT AND FUCOIDAN YIELD OF NEW ZEALAND NATIVE INTERTIDAL BROWN SEAWEED *Hormosira banksii* (TURNER) DECAISNE

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Culturing seaweed with bivalves and fishes has been shown to be economically efficient, as well as an effective method of bioremediation, to act as biofilters removing nutrients in the water from agricultural runoff that can give rise to toxic algal blooms. Seaweed also has many known and potential uses: in food, health, materials, research, pharmaceuticals and cosmeceuticals.

Pacific oysters already have a great reputation as a bioremediator, but it can be improved. Native seaweeds in New Zealand are only just beginning to be explored for commercial use. This study explores the feasibility of coculturing a native brown fucoid seaweed, *Hormosira banksii*, with Pacific oysters on an intertidal farm. This candidate seaweed was selected because it is an edible native littoral species, which has recently been shown to contain beneficial fatty acids and carbohydrate compounds. Studies have also shown its toughness and ability to withstand emersion periods, salinity and temperature fluctuations, and rough coastal conditions.

Growout potential of *H. banksii* on oyster farm infrastructure was assessed. The seaweed was sourced from six locations within two main areas, Mahurangi and Whangateau Harbours, local to the marine laboratory and oyster farm, and placed in oyster baskets for 200 days. Initial results indicate initial deployment mass and source location played a part in seaweed growth and survival, and improved methods are being devised.

Fucoidan is a variable polysaccharide found in brown seaweeds as well as some echinoderm species. It has been studied widely for anti-tumour, anti-angiogenesis, anti-inflammatory factors for pharmaceutical potential, and is used in cosmetics and human and stock feed supplements. It has not been previously shown to be found in this seaweed species.

Fucoidan was isolated from wild *H. banksii* from the same locations using the water extraction method adapted from Mak (2012). Fucoidan yield was found to have no significant difference based on whether they were from the Mahurangi or Whangateau ( $p = 0.881$ ), but significant differences were found based on the more specific locations each sample of seaweed was sourced from ( $p < 0.0005$ ). Improved cultivation methods and understanding the composition of fucoidan isolated from *H. banksii* and the effects of growout on fucoidan yield are being investigated using samples sourced from the most promising locations.



## RESULTS FROM A PARTICIPATORY ACTION RESEARCH FISH FEED TRIAL USING *Oreochromis macrochir* AND *Oreochromis tanganyicae* IN NORTHERN ZAMBIA

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Growth and water quality data collected systematically with farmers cultivating indigenous tilapia in earthen ponds in rural Zambia is non-existent. The lack of data is due in part because of inadequate human and financial resources but also the approach that many organizations use to implement and monitor and evaluate aquaculture activities in rural areas. WorldFish scientists and district Department of Fisheries (DoF) officers started implementing fish feed trials in 2016 with 41 fish farmers (20 females and 21 males) in Luwingu and Mbala Districts of Northern Province, Zambia as part of a larger research in development project funded by Irish Aid. Two indigenous tilapia species (*O. macrochir* and *O. tanganyicae*, respectively in the two districts) were stocked in farmers' ponds (10 x 10m) at a stocking density of 3 fish/m<sup>2</sup> testing three different treatments: feed only (maize meal + soy beans), manure only, and feed + manure. Growth monitoring was carried out monthly during the trials with each farmer, including the sampling of 30 fish/pond to measure weight and length as well as collecting water quality parameters (pH, dissolved oxygen, and temperature) over a six-month period. The trials were implemented using a participatory action research methodology that enabled WorldFish and DoF officers to collect the data while simultaneously developing capacities of rural farmers; many of whom had never received such extension support to date. Farmers participating in the feed trials were trained on formulating improved feeds, good management practices, and collecting growth and water quality data. Demonstration ponds were constructed and used as places for knowledge sharing and learning by farmers throughout the feed trials. This paper presents the growth and water quality trends over the course of the six-month trials, the first study of its kind to report such results of local tilapia species and pond water conditions in rural Zambia. The paper also details a relatively novel approach that research and development organizations can use not only to increase the likelihood of implementing successful aquaculture development projects, but also to collect important data that are missing from the extant literature on aquaculture in Zambia. This is important in the Zambian context given the recent growth in the aquaculture sector (driven mostly by larger-scale commercial farmers) and a desire by the Government and international donors to ensure aquaculture growth throughout the country is inclusive moving forward.

## AN EXPERIENCE PAPER: ALLER AQUA GROUP IN AFRICA; COMPANY STRATEGY AND CONTRIBUTION TO THE AFRICAN AQUACULTURE VALUE CHAIN

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Aller Aqua Group has been producing fish feed for fresh -and saltwater aquaculture for more than 50 years, and is therefore one of the most experienced fish feed producers. The company has achieved constant growth over the entire period, and has been expanding over the years. Aller Aqua Group has been delivering high quality extruded fish feed in Africa for more than a decade.

African aquaculture is constantly growing, and thus, the need for production optimization also increases throughout the value chain. Such development presents a series of challenges for the local aquaculture industry in the African continent.

As a response to this development, Aller Aqua Group implemented a new strategy for the African markets in 2015, focusing on the main challenges for the markets to the benefit of the entire value chain. Constant availability, economic conversion rate, high and constant quality, education and local presence as well as collaboration are just a few of the main challenges Aller Aqua addressed in the individual markets, and which are the fundamentals behind our strategy and business structure in Africa.

With factories, subsidiaries and distribution agreements in the main aquaculture nations in Africa, Aller Aqua presents a wide reach in the continent. This requires focus and support from a professional and dedicated organization – both locally and international.

With the investment in the most technologically advanced fish feed factory in Southern Africa – Aller Aqua Zambia Limited – Aller Aqua Group increases its presence and commitment to the aquaculture value chain on the African continent.



## GENETIC ANALYSIS OF SELECTED STRAINS OF PACIFIC ABALONE (*Haliotis discus hannai*) USING FISH AND MICROSATELLITE MARKERS

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The Pacific abalone, *Haliotis discus hannai* Ino, is one of most important aquaculture species in China. Artificial selection has been the common and inevitable approach in breeding. In present study, the effect of artificial selection on the genetic structure of two abalone lines (JJ selection strain, three successive selection lines based on the fast-growing trait, which was named JJF0, JJF1, JJF2, and JJF3; and R selection strain, the offspring of the red shell colored variants) were evaluated using 10 microsatellites. Loci showed from low to high polymorphism, with the number of alleles (A) ranging from 2 to 18. The mean observed ( $H_o$ ) and expected heterozygosities ( $H_e$ ) were  $0.650 \pm 0.022$  and  $0.711 \pm 0.018$ , respectively. In selection strain JJ, the values for most diversity genetic indexes (Ae, the number of effective alleles,  $H_o$  and  $H_e$ ) decreased from JJF0 to JJF3. Meanwhile, compared to JJ line, the genetic diversity estimates of R were close to those of JJF3. Nei's genetic distance ranged from 0.20357 to 0.51346. The unweighted pair group method with arithmetic mean (UPGMA) tree based on Nei's genetic distance also showed that the control Japan population and JJF0 formed to a cluster firstly, which were subsequently grouped together with JJF1, JJF2, JJF3, whereas the R was isolated from the rest of the populations. Analysis of genetic information indicated that genetic diversity was lost with artificial selection practices. Mechanisms underlying the maintenance of an acceptable level of genetic diversity while pursuing economic interests should be conducted in future research studies.

The karyotype of JJ selection strain has a common type  $2n = 36 = 10M + 8SM$  and two rare types  $2n = 36 = 11M + 7SM$  and  $2n = 36 = 10M + 7SM + 1ST$ . The results of silver staining showed that the NORs of JJ selection strain were located terminally on the long arms of chromosome pair 14 and 17 usually, the NORs were also located terminally on the short arms of chromosomes both metacentric pairs and submetacentric pairs. The 18s-rDNA FISH result was the first report about the location of 18s gene in *H. discus hannai*. FISH with 18S rDNA is consistent with results of silver staining. Highly polymorphic 18S rDNA locations occurred in *H. discus hannai*, they can be observed in the terminal of longor/and short arm chromosomes and submetacentric or/and metacentric chromosomes.

## POTENTIAL USE OF A CO-PRODUCT FROM THE BIOETHANOL PROCESS AS A PROTEIN SOURCE IN FEEDS FOR EUROPEAN SEABASS *Dicentrarchus labrax*

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The search for sustainable protein sources has become a major focus in the agriculture and aquaculture sector. Alternative sources such as plant proteins have been supplemented by a number of unique raw materials, such as insect meal, algal meals as well as co-products from industrial processes. The yeast protein concentrate (YPC) trialed in this study is a co-product from bio-ethanol processing using maize as a feedstock. The grain protein plus the spent yeast are extracted via a patented process to provide a potentially valuable feed protein containing up to 35% yeast.

Four extruded feeds (43% protein, 19% lipid, 6% ash and 23 MJ gross energy/kg) were based on fish meal and a mixture of plant-protein sources to supply equal amounts of digestible protein and energy. Fish meal inclusion was kept constant at 20% in all feeds and YPC was included at increasing levels of 4, 8 and 12% replacing sunflower meal and wheat gluten.

The trial was carried out in a recirculation system under controlled water quality parameters supplying three replicates per treatment. Fish grew from 35g to maximum 110g in 84 days; weight gain of seabass increased with increasing YPC concentration and was found to be significantly different at 8% and 12% levels compared to the control (zero YPC). Feed conversion ratio followed this trend and was significantly enhanced at the 8% and 12% YPC inclusion levels (Table 1).

To conclude, compared to the control diet, the inclusion of YPC in the diets was highly beneficial for growth and feed efficiency, particularly if included in the diets up to 12%, while voluntary feed intake was not affected.

**Table 1.** Performance parameters of seabass after 84 days of growth

| Treatment               | Control           | 4% YPC              | 8% YPC            | 12% YPC           |
|-------------------------|-------------------|---------------------|-------------------|-------------------|
| Weight initial (g)      | 35.0              | 35.0                | 35.0              | 35.0              |
| Weight final (g)        | 94.4              | 98.7                | 108.1             | 109.6             |
| Weight gain, g/day/fish | 0.71 <sup>a</sup> | 0.76 <sup>a,b</sup> | 0.87 <sup>b</sup> | 0.89 <sup>b</sup> |
| Feed intake, %/day      | 1.63              | 1.54                | 1.60              | 1.58              |
| FCR                     | 1.33 <sup>a</sup> | 1.19 <sup>a,b</sup> | 1.13 <sup>b</sup> | 1.10 <sup>b</sup> |

Different letters indicate significant differences at  $P < 0.05$

# HIGH-RESOLUTION LINKAGE MAPPING AIDED BY GENOME SURVEY AND TRANSCRIPTOME SEQUENCING IN *Portunus trituberculatus*: APPLICATIONS IN GROWTH-RELATED QTL AND GENE IDENTIFICATION

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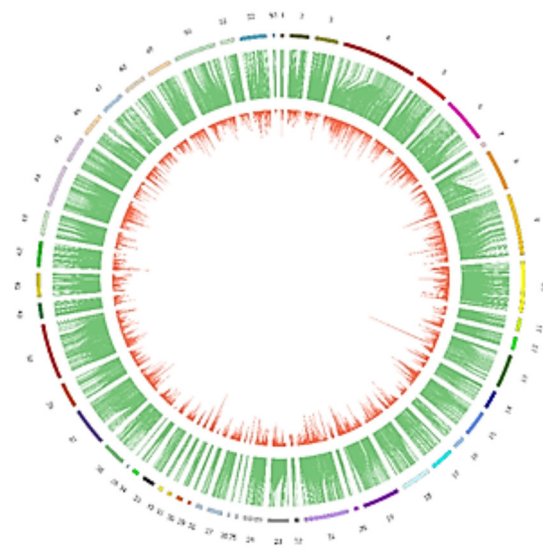
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A high-resolution genetic linkage map is an essential tool for decoding genetics and genomics in non-model organisms. In this study, linkage mapping was constructed for *Portunus trituberculatus* using specific-length amplified fragment sequencing (SLAF-seq). A high-resolution genetic linkage map with 10,963 markers was obtained, as far as we know, this has never been achieved in any other crustacean. The linkage map covered 98.85% of the whole genome with a mean marker interval of 0.51 cM.

A genome survey and transcriptome sequencing enabled 2,378 explicit annotated markers to be anchored to the map. Quantitative trait locus (QTL) mapping revealed 12 growth-related QTLs with a high mean *PVE* value of 23.7. Nine genes identified from the growth-related QTL region were considered important growth-related candidate genes. In particular, RE1-silencing transcription factor and RNA-directed DNA polymerase genes encoded nonsynonymous amino acids, which suggests a potential influence in growth regulation.

We have demonstrated that high-resolution linkage mapping aided by genome survey and transcriptome sequencing could serve as an important platform for QTL mapping and the identification of trait-related genes



**Fig Integration of linkage map, genomic scaffolds and transcripts.** Outer ring, the linkage group; Intermediate ring, contigs or scaffolds of genome assembly aligned with markers from the linkage map; Inner ring, transcript sequences of transcriptome aligned with markers of the linkage map.

## SUCCESSFUL BROODSTOCK DEVELOPMENT AND BREEDING OF PINK EAR EMPEROR *Lethrinus lentjan*, IN RECIRCULATION AQUACULTURE SYSTEM

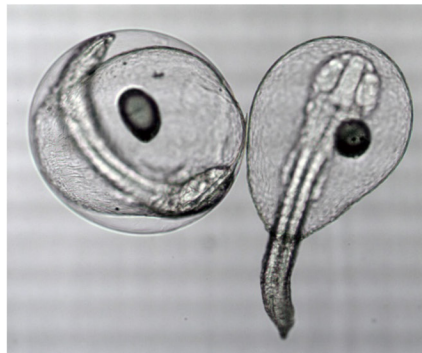
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Pink Ear Emperor *Lethrinus lentjan* (Lacepede, 1802) is a potential species for mariculture because to its high market value due to its superior flesh qualities, hardy nature and ability accept trash fish and compounded feed. Sixteen numbers of *L. lentjan* of the family Lethrinidae in the size range of 19 to 48 cm (750-1200g) were collected and stocked in 10 t RAS system and reared for a period of three months. The first successful spawning with fertilized eggs was obtained after three months rearing in RAS without any hormonal induction at Vizhinjam Research Centre of ICAR-CMFRI. The second spawning with more than 95% fertilization of eggs was obtained after a week and regular spawning was observed thereafter. The eggs hatched after about 16-18 hours after spawning at a water temperature range of 27-29° C. The newly hatched (pro larvae) larva measured 1100-1150 $\mu$ . At hatching, there was no mouth opening. The mouth opening was formed after about 30 hours post hatch. The mouth gape was around 110 $\mu$ . Larval rearing is done in green water feeding larvae with copepod nauplii, rotifer, artemia nauplii and micro diet. Juvenile stage with squamation is reached in 35-40 days.



*Lethrinus lentjan* brood stock



Hatchling



11 DPH larva



40 DPH juvenile



## SUCCESSFUL CAPTIVE BROODSTOCK DEVELOPMENT AND BREEDING OF SERRANID SEA-GOLDIE, *Pseudanthias squamipinnis* USING RECIRCULATION AQUACULTURE SYSTEM

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Sea-goldie, *Pseudanthias squamipinnis* (Peters, 1855) with vibrant pink shade belonging to subfamily Anthiinae (family: Serranidae) is a highly sought after marine ornamental fish. Successful captive brood stock development, spawning, and larval rearing are achieved at Vizhinjam Research Centre of ICAR-CMFRI. Brood stock was developed in a 5-ton recirculation aquaculture system (RAS) using 12 wild caught juveniles. Fishes were fed *ad libitum* using compounded feed and mussel meat. After 6 months rearing fishes (8-9.5 cm size) started courtship behavior such as vertical and slanting swimming and chasing. Spawning occurs at 1930 hrs. and fertilized eggs were collected using 250  $\mu$  mesh egg collector kept at the overflow conduit of the RAS. Eggs were of size 650  $\mu$ , transparent, pelagic, non-adhesive with single oil globule. About 4000 eggs at the stage of optic vesicle stage of embryo development were collected from the broodstock tank. They hatched out after an incubation period of about 12-14 hours at a water temperature of 29 °C. Newly hatched larvae measured 1400  $\mu$  and were stocked in 1-ton larval rearing tanks at the rate of 2 larvae per litre filled with green water at a density of  $1 \times 10^6$  cells per ml using *Nannochloropsis oculata* and *Isochrysis galbana* at 1:1 ratio. Mouth opened at 40-50 hours post hatch and measured 50-70  $\mu$  and the total length of larvae was 1786  $\mu$ . Larvae were fed with copepod nauplii (40-60  $\mu$ ) at the rate of 10 nauplii per 50 ml, from 3 DPH and co-feeding with enriched artemia from 17 DPH at the rate of 0.2/ml. By 40<sup>th</sup> day they reached the juvenile stage with a survival rate of 4% and at which they were transferred to nursery rearing tank.

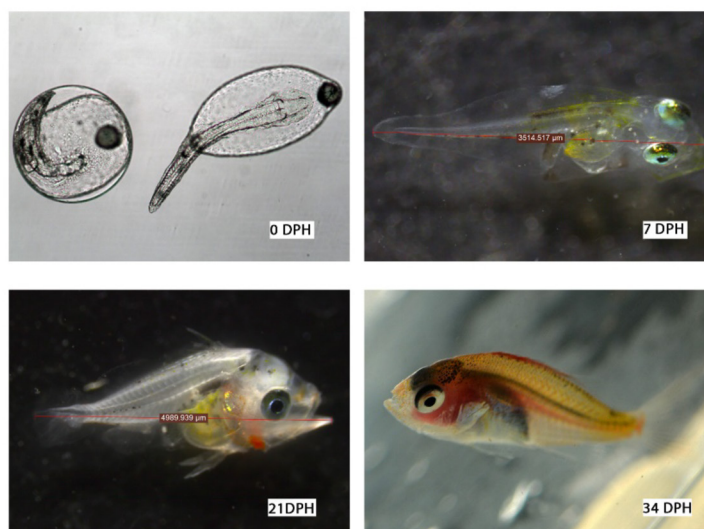


Fig. 1. Larval development stages of *Pseudanthias squamipinnis*



## DEVELOPMENT OF AN EGG DISINFECTION PROTOCOL FOR SOUTH AFRICAN MARINE AQUACULTURE FINFISH SPECIES

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As the South Africa local mariculture finfish industry is still in its infancy, the originally anticipated consistent availability of eggs within individual facilities has not been forthcoming but instead has been frustratingly sporadic. The transfer of disease via eggs has been demonstrated in various cultured fish species suggesting that it is highly possible that pathogens could be transferred between facilities with potential catastrophic consequences for the industry. Adoption of an egg disinfection protocol by hatcheries would serve to mitigate the potential transfer of disease between facilities and minimize the vertical transfer of disease within a facility.

The aim of this study was to develop a safe, simple and effective egg disinfection protocol for South African mariculture finfish species which can be implemented at source, thereby significantly reducing the potential transfer of egg-born disease between southern African mariculture/research facilities. Dusky kob (*Argyrosomus japonicus*) and Yellowtail (*Seriola lalandi*) eggs were obtained from captive broodstock fish held at Pure Ocean (Pty) Ltd, East London. Broodstock were conditioned to spawn in a partial flow through recirculating system.

The first part of the study was to determine the efficacy of four disinfection agents on their ability to adequately reduce the bacteria microflora community on the surface of dusky kob eggs. Four disinfection agents (Formaldehyde (FO: 2.5g/L for 15 min & 5g/L for 5min), Povo-iodine (PI: 5g/L for 15min & 10g/L for 5min), Chloramine-T (CT: 7.5g/L for 10min & 15g/L for 5min), Hydrogen peroxide (HP: 1.8g/L for 15min & 0.9g/L for 5min) & untreated control were tested at various concentrations and exposure times. The untreated egg control had a (mean $\pm$ SE 786.5 $\pm$ 118.5 CFU/mL). Doses with higher concentrations and five minute exposure times showed greater efficacy. All disinfectants with the exception of formaldehyde significantly ( $P<0.05$ ) reduced the bacterial microflora community on the surface of the eggs (CT15g/L 5min 8 $\pm$ 0; CT7.5g/L 10min 9.5 $\pm$ 0.5; HP1.8g/L 5min 19.3 $\pm$ 7.8; HP0.9g/L 15min 52 $\pm$ 3; PI10g/L 5min 21.3 $\pm$ 4.8 & PI5g/L 15min 24.5 $\pm$ 5.5 CFU/mL). The best two disinfectants (Chloramine-T & Hydrogen peroxide) will be tested to determine the effect of the disinfecting agent on the hatchability, survival and quality (i.e. deformities) of dusky kob larvae. Once the optimal regime had been identified based on efficacy, simplicity of use and cost, the regime will be tested under commercial conditions.

## THE EFFECT OF CARBOHYDRATE ON DUSKY KOB *Argyrosomus japonicus* FED PELLETTED DIETS

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The dusky kob, *Argyrosomus japonicus*, is an emerging commercial marine aquaculture species in South Africa. Feed is a limiting factor in dusky kob production as its nutritional requirements are not well understood. The approximate protein and energy requirements for kob have been established in earlier research, but the ability of kob to utilize carbohydrates as an energy source has not previously been investigated. The aim of this study was to determine the efficiency of dusky kob to utilize dietary carbohydrate in pelleted feeds. The objectives were to determine the effect on i) growth performance and feed utilization, ii) post prandial blood glucose and iii) the health of dusky kob fed diets containing different levels of pregelatinized maize starch (PGMS).

Juvenile dusky kob (5g) were acclimatized in a recirculating aquaculture system Isoenergetic, isonitrogenous diets containing 4.1, 8.2, 16.4 and 24.6% carbohydrate, were formulated with pregelatinized maize starch as a carbohydrate source and fishmeal as the main protein source. Fish were fed twice daily at 3.85% body weight/day for three months.

Specific growth rates and feed conversion ratios differed significantly between the four dietary treatments. Growth rate increased with increasing carbohydrate up to 16.4%, after which a decline was observed. The diet with 16.4% carbohydrate produced the highest specific growth rate (SGR) of  $1.84 \pm 0.05\%$  body weight/day, and the lowest feed conversion ratio (FCR) of  $1.28 \pm 0.08$ . While the lowest SGR and FCR were found in the diet with 24.6% carbohydrate. A third order polynomial regression, using SGR, determined the optimum carbohydrate inclusion for dusky kob to be 16.72%.

The general structure of the liver was similar between all fish fed the dietary treatments and all fish liver samples displayed a certain degree of lipid vacuolization of the hepatocytes. Evidence of starvation was observed in the livers of fish fed diet with 24.6% carbohydrate. No differences in the amount of liver glycogen were observed.

Gut bacterial composition did not differ among the different diets or between the different sections of the gut. Differences were however observed in the diversity of the bacterial community structure at the start (when they were fed commercial trout feed) and end of the experimental period

The results demonstrated dusky kob effectively utilize carbohydrate up approximately 20% inclusion level, above which growth performance, feed conversion efficiency and health decline.

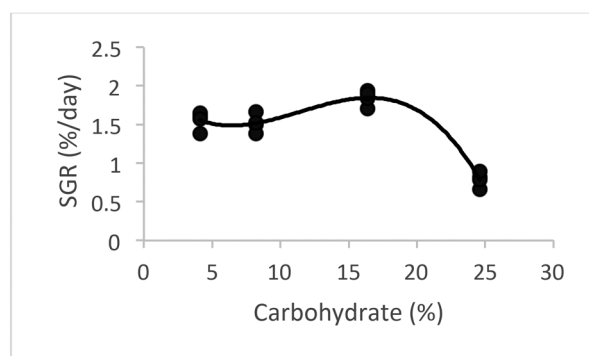


Figure 1: Relationship between specific growth rate (SGR, %/day) and dietary carbohydrate level in juvenile dusky kob ( $y = -0.0007x^3 + 0.0239x^2 - 0.2213x + 2.1046$ ,  $R^2 = 0.94$ ).

## **ENFORCING AQUACULTURE REGULATIONS UTILITIZING UNDERWATER DRONES (ROVs)**

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The proposed presentation offers a case study analysis of the use of Deep Trekker mini-ROVs to perform regulatory compliance inspections.

### **Reason**

Illegal aquaculture sites pose a significant risk to both to the environment and the surrounding communities. Monitoring the areas of high risk is extremely difficult due to the time, risk and costs associated with using divers. Remotely operated vehicles or 'underwater drones' have become the new tool for enforcement officers to quickly inspect areas of interest.

### **Cases**

An in depth look at how regulatory officers are currently using underwater drones in their daily practice, what sort of discoveries have been made, and ways to properly document the underwater environment to adhere to environmental regulations.

### **Conclusions**

Findings suggest that the use of ROVs have significantly improved officer's ability to accurately patrol regions of interest and document findings. With ROVs officers can quickly act to gather information underwater without bringing in divers.

Regulations in the aquaculture industry are continuing to increase to properly monitor and minimize environmental impacts. These case studies showcase how underwater drones can improve the accuracy of inspections and ensure detrimental practices, such as illegal aquaculture sites are quickly identified.

## MINI-ROVS IMPROVING AQUACULTURE OPERATIONS

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Co-Author: John Wintermeyer

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Aquaculture operations involve a number of tasks being handled on a daily basis to ensure farm integrity. These tasks include but are not limited to net inspections, mort retrieval, size grading, and fish monitoring. The proposed presentation offers a case study analysis of the use of Deep Trekker mini-ROVs to perform daily inspection tasks at Canadian salmon aquaculture sites.

### Reason

Salmon farms require divers to perform tasks and inspections but associated costs can be high. Mini-ROVs are revolutionizing the way that managers go about their daily operations without hiring divers until there is a true need to have hands below the water. Mini-ROVs are able to perform net inspections, observe fish behaviour and bridge the knowledge gap between employees and what takes place below the surface while simultaneously recording the footage.

### Cases

The case studies include 2 Canadian aquaculture companies; Marine Harvest and Aqua-Cage Fisheries to demonstrate the ways that a mini-ROV can improve operation efficiency and reduce costs.

### Conclusions

Findings suggest that the use of mini-ROVs have significantly reduced farm managers' needs to hire dive teams. With their ROVs, Agrimarine and Marine Harvest can perform daily inspections and ensure that there's an issue before calling in divers.

If infrastructure integrity is compromised, the faster a problem is identified the sooner it can be fixed, improving efficiency and reducing loss. Mini-ROVs can be deployed immediately, eliminating the need to call divers and wait for them to arrive. In cases where net integrity is compromised, hasty inspection is key to ensuring zero loss.

Dropped equipment is either ignored because costs associated with retrieving it are not worth it or divers are called and costs incurred. With ROVs, managers and employees have been able to retrieve equipment without the need to call help.

The use of mini-ROVs has given managers the ability to teach employees about the underwater aspects of the farm as well as identify and explain any problems to a dive team in cases where divers are needed.

**Biography:** Sam MacDonald has been working alongside the Aquaculture industry with Deep Trekker Inc., visiting farms, doing demos and exploring cages with mini-ROVs. The ROVs are being used in over 70 countries across the world to improve aquaculture operations in a variety of ways. She has written numerous pieces about the use of ROVs in the industry and produced several short videos.

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Category: Oral Presentation



## REQUIREMENT OF DIETARY $\alpha$ -LINOLENIC FATTY ACID FOR JUVENILE NILE TILAPIA AT COLD SUBOPTIMAL TEMPERATURE

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There are gaps in the knowledge about fatty acid nutritional requirements of Nile tilapia, particularly when raised in suboptimal temperature. The objective of this study was to estimate the dietary requirement of  $\alpha$ -linolenic acid ( $\alpha$ -LNA, 18:3n-3) for Nile tilapia, when raised at 22°C. Increasing levels of linseed oil were added to a mixture of vegetable oils, yielding the following concentrations of  $\alpha$ -LNA: 0.03, 0.21, 0.37, 0.67, and 0.99% dry weight. The basal diet was formulated with semipurified ingredients and contained 5% lipid. Juvenile tilapia ( $10.6 \pm 0.28$  g) were fed the experimental diets twice daily until satiation for 14 weeks. The average water temperature throughout the experimental period was  $22.12 \pm 0.17^\circ\text{C}$ .

The increase in dietary  $\alpha$ -LNA significantly affected weight gain, specific growth rate, feed efficiency and daily feed intake. Total n-3 fatty acid content in muscle showed a significant linear response to the increasing dietary  $\alpha$ -LNA. Likewise, an accumulation of muscle linoleic acid (18:2n-6) was directly related to the increase in dietary  $\alpha$ -LNA; however, total n-6 long-chain polyunsaturated fatty acids (LC-PUFA) content in the muscle decreased.

The dietary requirement of  $\alpha$ -LNA - estimated for juvenile Nile tilapia from 10.67 to 59.80 g - was 0.70% for a maximum weight gain of  $49.13 \text{ g day}^{-1}$  and 0.68% for maximum feed efficiency.

## GROWTH AND ECONOMIC PERFORMANCE OF CATFISH JUNDIÁ *Rhamdia quelen* FED PRACTICAL DIETS WITH DIFFERENT ENERGY:PROTEIN RATIOS AND FORMULATED ON A DIGESTIBLE-AMINOACID BASIS

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We assessed growth and economic performance of juvenile catfish jundiá, *Rhamdia quelen* ( $31.54 \pm 4.92$  g body weight), fed diets formulated based on digestible aminoacids of practical ingredients. Different levels of digestible protein (DP, 24 to 44%) and digestible energy (DE, 2900 to 3600 kcal.kg<sup>-1</sup>) were tested with the following digestible energy:protein ratios (DE:DP): 12, 11, 10, 9, and 8 kcal.g<sup>-1</sup>. An extra lipid-rich diet (29% DP, 3500 kcal.kg<sup>-1</sup> DE; DE:DP = 12 kcal.g<sup>-1</sup>) was also included in order to verify if there is protein sparing effect when juvenile jundiá is fed diets rich in non-protein energy. Each practical diet was offered to three groups of 25 fish, for 75 days and fish were fed twice a day (0900 and 1600h).

The regression analyses showed that the DE:DP ratio significantly affected all growth and economic performance variables, as well as the total-ammonia-nitrogen content in the water which increased as the dietary protein and energy increased. Body fat deposition followed the decrease in dietary DE:DP. The inclusion of 12% lipid in the extra lipid-rich diet did not compromised fish growth significantly when compared to fish fed diets containing 34% or 39% DP – with 8% lipid content – but the extra lipid inclusion significantly reduced body protein deposition.

According to the regression models of weight gain and feed conversion, the present study suggests for juvenile jundiá a dietary DE:DP ratio of 9.08 kcal.g<sup>-1</sup> (38.28% DP and 3437.5 kcal.kg<sup>-1</sup> DE) and 9.58 kcal.g<sup>-1</sup> (36.07% DP and 3125 kcal.kg<sup>-1</sup> DE), respectively. However, considering the regression models of protein retention and feeding cost, the best dietary DE:DP ratios for juvenile catfish jundiá are 10.34 kcal.g<sup>-1</sup> (32.29% DP and 3229 kcal.kg<sup>-1</sup> DE) and 10.4 kcal.g<sup>-1</sup> (33.38% DP and 3200 kcal.kg<sup>-1</sup> DE), respectively.

THE EPIDEMIOLOGY OF ULCER DISEASE OF ATLANTIC SALMON *Salmo salar* IN ATLANTIC CANADA

Brett MacKinnon\*, Henrik Stryhn, Jenny Yu, Raphael Vanderstichel, and Sophie St-Hilaire

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Ulcer disease is the main reason for antibiotic usage in Atlantic salmon (*Salmo salar*) aquaculture in Atlantic Canada and identification of risk factors and management strategies for this disease are needed to improve the sustainability of the industry. *Moritella viscosa* is hypothesized to be the cause of the outbreaks, but this is unconfirmed since it is not always isolated from field samples. The objectives of this study were to describe ulcer disease cases at the cage and farm levels to determine potential sources and transmission of infection, and identify factors that could potentially affect the expression of clinical disease.

Weekly sea cage-level mortality, medical records, and data on management practices from 29 Atlantic salmon aquaculture farms in the Atlantic Canada region, operating between 2014 and 2016, were extracted and summarized. The onset, duration, and total mortality associated with the ulcer disease outbreak time-periods were determined at the cage and farm levels. The spatial and temporal patterns of ulcer disease outbreaks at the farm-level were investigated using QGIS. The potential effect of risk factors (such as sea lice treatments and cage density) on the total mortality rate during the outbreak (outcome) was assessed using a mixed-effects linear regression model (including the random effects of farm).

There were 12 farms diagnosed with ulcer disease, with a total of 95 sea cages on affected farms. There was a relatively simultaneous start of ulcer disease at the cage-level, suggesting a common point source exposure to the causative agent of ulcer disease. There were unaffected cages on most farms with the disease, which is not consistent with cage-to-cage transmission of the pathogen. There was a large range in the severity of outbreaks at the cage-level. At the farm-level, there was a similar temporal pattern of disease for 2014 and 2015, suggesting that the pathogen is widespread from mid-summer to mid-fall or outbreaks are triggered by an environmental factor. The final model had three statistically significant predictors for ulcer disease mortality (Table 1).

Table 1. Results of the final mixed-effects linear regression model with natural log-transformed outcome (total percent mortality during the outbreak time-period).

| Variable  | Coefficient | 95% CI       |
|---|-------------|--------------|
| Was there a diagnosis of ulcer disease at the cage-level? |             |              |
| No  | Ref.        |              |
| Yes   | 0.887       | 0.422, 1.353 |
| Antibiotic treatment during the outbreak time-period      |             |              |
| None  | Ref.        |              |
| Oxytetracycline or florfenicol                            | 2.210       | 1.543, 2.876 |
| Tribissen   | 3.583       | 2.730, 4.435 |
| Year class of saltwater entry                             |             |              |
| 2014  | Ref.        |              |
| 2015  | 1.930       | 1.040, 2.820 |



## DIGESTIVE ENZYMES OF SIBERIAN SYMPATRIC PAIR OF WHITEFISH (*Coregonus sp.*): FUNCTIONAL AND STRUCTURAL ANALYSIS

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*Coregonus lavaretus* – is widely spread species of whitefish in the north hemisphere that may form different sympatric pairs in lakes. One of such pair inhabits in Lake Teletskoye. The sympatric pair is formed by *Coregonus lavaretus pidshian* with less than 30 gill rakers on the first brachial arch and *Coregonus pravdinellus* that has more 30 (average is 34) ones. *C. l. pidshian* is zoobenthivorous whereas *C. pravdinellus* is zooplanktivorous. Both of them are very important source of animal protein for natives and very interesting model to study the sympatric evolution process. The main aim of the study was to compare activity of digestive enzymes in different parts of fish gut and make their structural identification.

The fish were caught in Lake Teletskoye (51°79'N; 87°26'E, Altai region, Russia) by nets with mesh size 20-40 mm. After their capture, the fish were dissected and the guts were extracted and frozen in liquid nitrogen until analyze. The activities of alpha-amylase, lipase, non-specific esterase, total alkaline proteases, trypsin, chymotrypsin, carboxypeptidase A and B, aminopeptidase and alkaline phosphatase were assayed. For structural identification of proteins the anterior part of intestine was chosen. For separation of proteins the combination of ion-exchange and hydrophobic high pressure liquid chromatography was conducted and then the collected peaks were run on SDS-PAGE electrophoresis. Then, all obtained bands of protein were transported on PVDF Immobilon SQ membrane and the sequencing of N-terminal aminoacid (15 residues) was done.

The activity of all studied enzymes had the similar trends throughout the all parts of intestine of *C.l. pidshian* and *C. pravdinellus* but the level of activities was different. Thus, the activities of trypsin, chymotrypsin, carboxypeptidase A, alpha-amylase were higher in anterior and middle intestine of *C.l. pidshian*. We extracted and determinated the following enzymes: trypsin-, chymotrypsin- and subtilisin-like enzymes, phospholipase like and leucine-aminopeptidase.

## DEVELOPMENT OF A CARRYING CAPACITY MODEL FOR SUSTAINABLE CAGE AQUACULTURE IN LAKE MALAWI/NIASSA/NYASA

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The carrying capacity of a tilapia cage aquaculture farm in the South East Arm (SEA) of Lake Malawi was estimated between December 2011 and September 2012 during the day and night periods at six sampling sites along the farm perimeter to investigate spatial and temporal changes in dissolved oxygen (DO) concentrations owing to oxygen demand by the fish farm.

Significantly higher night DO concentrations were recorded in March, May, and June in all sites and in February, April, and August in few sites ( $p < 0.05$ ) while significantly higher DO concentrations during the day were observed in July and September ( $p < 0.05$ ). Nearshore sites seem to be more oxygenated than offshore sites within the farm, since they had relatively higher DO than offshore sites in most months. However, the surface waters had DO above 6.5 mg/l most of the time, except in June and July, but even then always higher than the minimum recommended values. A full hydrodynamic model is needed to confirm current observations.

The sub-model of water quality found that oxygen consumption of fish at the cages, oxygen transport, and carrying capacity (CC) were variable and showed no pattern with regard to positioning of the cage (i.e. nearshore-offshore) at the farm. None of the cages were overstocked and the CC was much higher than maximum existing biomass at the cages (18,824 kg). To allow normal fish growth rates and attainment of desirable marketable, mean stocking density of  $23,389 \pm 44$  kg (Figure 1a) and  $1,866,689 \pm 95,678$  kg (Figure 1b) are recommended for individual cages) and for the entire Maldeco Aquaculture farm respectively.

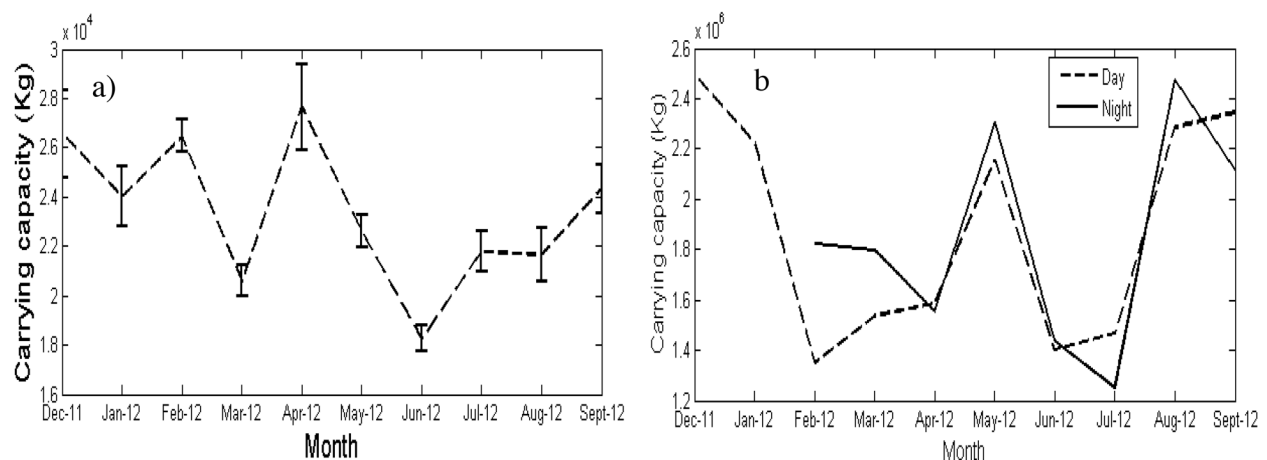


FIGURE 1. Mean cage (a) and Farm (b) carrying capacity estimates based on individual cage and perimeter observations respectively.

## EARTHWORM MEAL AS PROTEIN SOURCE IN NILE TILAPIA DIETS

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Tanzania has experienced dwindling fish catch due to overfishing and environmental degradation resulting in annual yield of about 350,000 MT which is half of the potential. This has resulted in low per capita fish consumption of 8 kg, far less than the global average of 20 kg. On the other hand, aquaculture has remained subsistence practiced in small semi-intensive freshwater ponds which are mostly stocked with Nile tilapia. Lack of quality affordable aquafeeds is one of the limiting factors as fishmeal and oil seed cakes which are main sources of protein are scarce and costly. Earthworms are locally available and relatively affordable and have high protein content, thus potential alternative protein source. This study was conducted to evaluate earthworm meal (EWM) as alternative protein source in practical aquafeeds. The earthworms were produced using cow manure as substrate. Five isonitrogenous with 30% crude protein were formulated to contain graded levels of EWM (0, 12, 24, 40 & 45%) as shown in Table 1. Each diet was randomly assigned in triplicate to experimental units containing 14 tilapia juveniles each with an average weight of 2.6 g. The diets were fed for eight weeks collecting data on body weights and feed intake. Best growth, feed utilization and cost effectiveness was observed in fish fed diet EWM40 (Table 2). Therefore, EWM can be included at 40% in Nile tilapia diets containing 5% fish meal and 5% cotton seedcake without compromising performance.

**Table 1: Percentage inclusion levels of the ingredients in EWM Based Diets (g/100g diet)**

| Ingredients      | Diets |       |       |       |       |
|------------------|-------|-------|-------|-------|-------|
|                  | EWM0  | EWM12 | EWM24 | EWM40 | EWM45 |
| Fish Meal        | 5.0   | 5.0   | 5.00  | 5.0   | 5.0   |
| Earthworm meal   | 0.0   | 12.0  | 24.0  | 39.8  | 45.0  |
| Cotton seed meal | 50.0  | 38.0  | 24.5  | 5.0   | 0.0   |
| Others*          | 45.0  | 45.0  | 46.5  | 50.2  | 50.0  |
| Total            | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

\*Maize meal, wheat meal, sunflower oil and Vitamin/Mineral premix

**Table 2: Performance of Nile tilapia fed EWM diets (Mean  $\pm$  SD, n=3).**

| Parameter                                   | Diets                           |                                |                                  |                                 |                                 |
|---|---------------------------------|--------------------------------|----------------------------------|---------------------------------|---------------------------------|
|   | EWM0                            | EWM25                          | EWM30                            | EWM35                           | EWM40                           |
| IBW (g)                                     | 2.41 $\pm$ 0.18 <sup>a</sup>    | 2.48 $\pm$ 0.05 <sup>a</sup>   | 2.42 $\pm$ 0.08 <sup>a</sup>     | 2.43 $\pm$ 0.05 <sup>a</sup>    | 2.46 $\pm$ 0.40 <sup>a</sup>    |
| FBW (g)                                     | 7.71 $\pm$ 0.071 <sup>c</sup>   | 8.50 $\pm$ 0.28 <sup>b</sup>   | 8.84 $\pm$ 0.48 <sup>a</sup>     | 8.92 $\pm$ 0.06 <sup>a</sup>    | 7.71 $\pm$ 0.02 <sup>c</sup>    |
| BWG   | 5.30 $\pm$ 0.25 <sup>c</sup>    | 6.02 $\pm$ 0.25 <sup>b</sup>   | 6.42 $\pm$ 0.44 <sup>a</sup>     | 6.49 $\pm$ 0.10 <sup>a</sup>    | 5.25 $\pm$ 0.38 <sup>c</sup>    |
| ADWG (gday <sup>-1</sup> )                  | 0.096 $\pm$ 0.004 <sup>d</sup>  | 0.104 $\pm$ 0.005 <sup>c</sup> | 0.115 $\pm$ 0.008 <sup>b</sup>   | 0.118 $\pm$ 0.002 <sup>a</sup>  | 0.096 $\pm$ 0.007 <sup>d</sup>  |
| FI (gfish <sup>-1</sup> day <sup>-1</sup> ) | 0.22 $\pm$ 0.01 <sup>c</sup>    | 0.24 $\pm$ 0.01 <sup>ab</sup>  | 0.26 $\pm$ 0.01 <sup>a</sup>     | 0.28 $\pm$ 0.01 <sup>a</sup>    | 0.23 $\pm$ 0.01 <sup>c</sup>    |
| FCR   | 2.47 $\pm$ 0.14 <sup>c</sup>    | 2.22 $\pm$ 0.07 <sup>b</sup>   | 2.10 $\pm$ 0.09 <sup>a</sup>     | 1.85 $\pm$ 0.31 <sup>a</sup>    | 2.43 $\pm$ 0.04 <sup>c</sup>    |
| SGR   | 2.11 $\pm$ 0.15 <sup>c</sup>    | 2.26 $\pm$ 0.05 <sup>b</sup>   | 2.31 $\pm$ 0.08 <sup>a</sup>     | 2.38 $\pm$ 0.05 <sup>a</sup>    | 2.18 $\pm$ 0.31 <sup>c</sup>    |
| PER   | 1.35 $\pm$ 0.01 <sup>c</sup>    | 1.57 $\pm$ 0.05 <sup>c</sup>   | 1.80 $\pm$ 0.21 <sup>b</sup>     | 1.81 $\pm$ 0.07 <sup>a</sup>    | 1.68 $\pm$ 0.19 <sup>b</sup>    |
| Surv (%)                                    | 88.1 $\pm$ 8.6 <sup>a</sup>     | 97.6 $\pm$ 2.4 <sup>a</sup>    | 97.6 $\pm$ 2.4 <sup>a</sup>      | 97.6 $\pm$ 2.4 <sup>a</sup>     | 92.9 $\pm$ 0.0 <sup>a</sup>     |
| CF (TZS/Kg)                                 | 1509                            | 1419                           | 1419                             | 1434                            | 1449                            |
| CE (TZS/Kg fish)                            | 3727.2 $\pm$ 215.9 <sup>d</sup> | 3150.2 $\pm$ 7.6 <sup>b</sup>  | 2979.8 $\pm$ 348.7 <sup>ba</sup> | 2659.8 $\pm$ 104.7 <sup>a</sup> | 3521.1 $\pm$ 280.4 <sup>c</sup> |

IBW=Initial body weight, FBW=Final body weight, BWG=Body weight gain, ADWG=Average Daily Weight Gain, FI=Feed Intake, FCR=Feed Conversion Ratio, SGR=Specific Growth Rate, PER=Protein Efficiency Ratio, Surv=Survival, CF=Cost of feed, CE=Cost effectiveness.

Means with different superscript letters within a row are significantly different at P<0.05

## GROWTH, HEAMATOLOGY, AND GUT HISTOLOGY OF JUVENILE DUSKY KOB (*Argyrosomus japonicus*) OFFERED SEAWEED (*Ulva sp.*)-BASED DIETS

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The high cost of feed is a major constraint to make profitable marine finfish aquaculture in the coastal regions of South Africa. The current study was designed to assess the effect of graded levels of green macroalgae seaweed, *Ulva sp.*, on growth, blood parameters and gut histology when incorporated into diets of juvenile Dusky kob, *Argyrosomus japonicas*. Five isonitrogenous (45% CP) experimental diets were formulated to contain 0, 50, 100, 150 and 200 g/kg seaweed on dry matter basis. Seventy-seven Dusky kob fingerlings (mean body mass  $9.14 \pm 0.030$  g) were distributed into twenty recirculating, 465 L black high density polyethylene tanks, which were divided into four replicates per treatment. Fish were fed at a rate of 2.8% of average body mass and weighed weekly for nine weeks. Fish fed 50 g/kg *Ulva* diet had higher body mass compared to those fed the other three *Ulva* supplemented diets (figure 1). The 200 g/kg *Ulva* diet fed group grew significantly slower compared to the rest of the groups, with some fish evidently losing weight. Increasing the levels of dietary seaweed reduced the specific growth rate of fish, with the control diet promoting the highest (2 g/day). Effect of the dietary treatments on growth performance was significantly different from the second week until termination ( $P < 0.05$ ). There was no difference ( $P > 0.05$ ) between dietary treatments with respect to haematological parameters. Gut histology preparations revealed normal gut structure for all the dietary treatments. Zero intestinal cellulase activity was recorded. In conclusion, *Ulva* supplementation beyond 50 g/kg is not recommended in dusky kob diets as this leads to reduced growth performance.

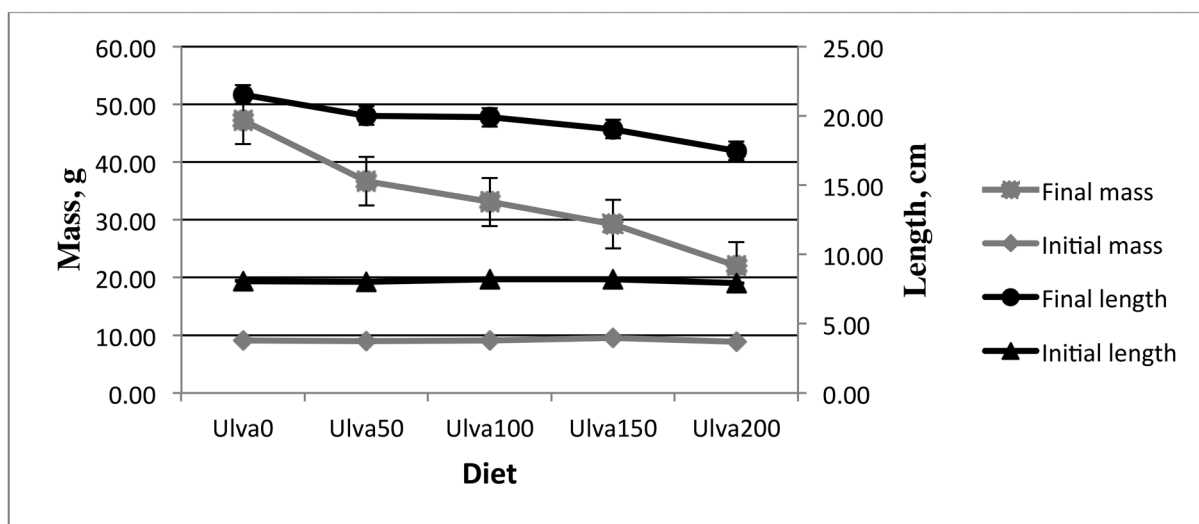


Figure 1. Variations in gain of mass and length parameters in response to dietary treatments. Data points represent means of four replicates  $\pm$  standard error.

## AN ASSESSMENT OF KNOWLEDGE TRANSFER SYSTEMS TO FRESH WATER AQUACULTURE FARMERS IN KWAZULU-NATAL (KZN)

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Researchers generate large volumes of information on aquaculture but where does the bulk of it end? South Africa has 26 Universities, 10 of which offer aquaculture or marine biology studies (FAO, 2016). The knowledge generated may not necessarily be efficiently transferred to the end user. Thus, there is an uncoupling between aquaculture research and the aquaculture production in South Africa. Aquaculture is one of the growing food production sectors in the world, yet Africa and South Africa only contribute 2 % and 0.1% to the total respectively. Hence, the assessment of knowledge transfer to fresh water aquaculture farmers is of academic interest to researchers; business interest to the farmer, Government, conservation organisations and others.

Instead of focusing on the evaluation of knowledge transfer interventions in aquaculture most of the available research and literature tend to focus on aquaculture technology (Ahmed *et al.*, 1995). It is important to close the knowledge gap by giving feedback to the researcher with regard to how much of the information generated is transferred and adopted by the farmer (Ashley-Dejo *et al.*, 2013). This research seeks to investigate the knowledge transfer process in aquaculture to provide a deeper understanding and a holistic analysis of the factors that facilitate and/or inhibit knowledge transfer and adoption to fresh water aquaculture farmers in KZN. In particular, the main focus will be on answering three questions namely: How is knowledge transferred from the researcher to the fresh water fish farmer in KZN? To what extent is transferred knowledge adopted/ utilized? Which factors facilitate and/or inhibit knowledge transfer and adoption.

This survey will take on a mixed approach; hence both qualitative and quantitative questions will be explored. As a result, a variety of data collection methods will be used. These include: questionnaires, farm operation observations and interviews. This study will employ the social interaction models which emphasize knowledge diffusion between persons and systems. According to these models the utilization of knowledge is a result of repeated interactions between researchers and end users who are considered as co-producers of knowledge. The interaction will probably improve the relationship between research and the fresh water fish farmers and thereby also improve both transfer and adoption of new knowledge/technology, and increased productivity. Understanding factors that facilitate or inhibit knowledge transfer and adoption can guide further research and improve knowledge transfer and adoption. This paper is only limited to fresh water and does not include marine aquaculture. Below are the results in table 1.

**Table 1: Top three Systems/Modes of knowledge transfer and factors facilitating/inhibiting both knowledge/technology transfer and adoption in KZN**

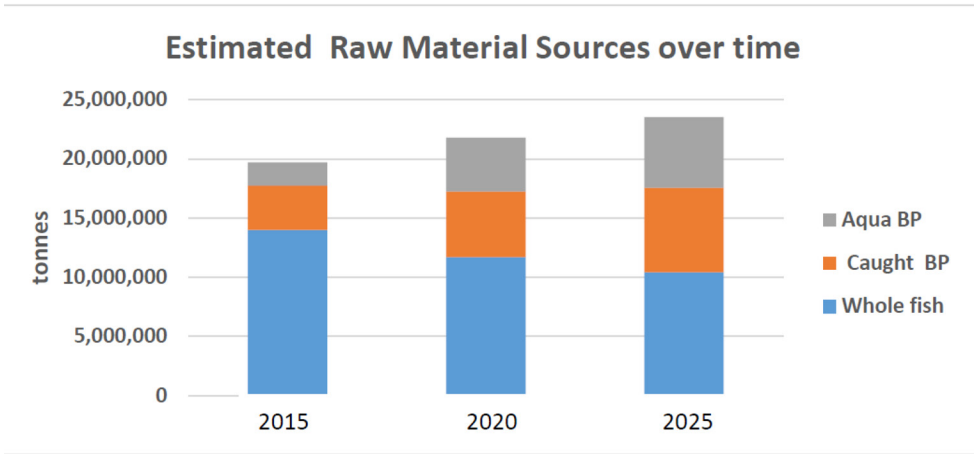
| System/Mode      | Percentage | Adoption rate | Factors facilitating/inhibiting knowledge transfer/ adoption |
|------------------|------------|---------------|--|
| Internet         | 78         | 33            | Knowledge related  |
| Farmer to Farmer | 78         | 45            | Source related   |
| Magazine         | 78         | 22            | Recipient related  |
| Average          | 78         | 33.3          | Relationship related   |
|                  |            |               | Mode of transfer related                                     |

**MARINE INGREDIENTS IN AQUACULTURE FEEDS – AVAILABILITY, SUSTAINABILITY AND CONTRIBUTION IN FUTURE**

A Mallison

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Marine ingredients in feed have been the foundation of the modern aquaculture industry. To date, they remain the most nutritious and digestible sources of protein, oil and micro-nutrients for farmed fish. However, availability from traditional sources is capped by careful fishery management and the need to provide whole fish for direct human consumption is taking species that traditionally were available for marine ingredient manufacture to other markets. This presentation addresses the concerns about availability, presents new research on the potential of raw materials from by-products and progress towards responsible fishery management.



## INTERGRATED AQUACULTURED BASED ON SUSTAINABLE WATER RECIRCULATING SYSTEM FOR THE VICTORIA LAKE BASIN (VICINAQUA)

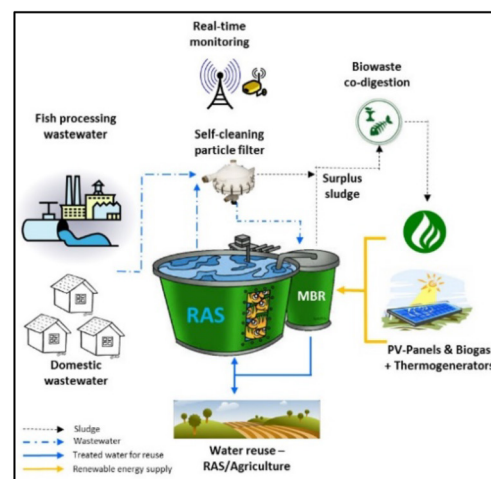
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Lake Victoria is a the second largest freshwater lake in the world and of key socio-economic importance for the region, supporting a population of around 30 million through large scale fishing, agriculture, tourism and other local industries. Despite its crucial importance, Lake Victoria has suffered the consequences of overexploitation of its resources (mainly fish stocks) and alarming pollution. One of the main challenges around the lake is poor sanitation and water provision infrastructure and measures and solutions on water supply and sanitation are urgently required.

VicInAqua, a project under the Horizon 2020 research and innovation programme, will develop innovative multipurpose self-cleaning water filtration solutions adapted for sanitation of different wastewater systems to be reused in Recirculation Aquaculture Systems (RAS) around Lake Victoria. The main goal of this project is to enable the supply of clean water to RAS and agriculture through a single solution for water treatment of different waste water streams (domestic waste, fish production and processing industry). The system will have a fully autonomous power supply by renewable energy (PV, biogas, solar) and remotely monitored with sensor technologies. The technology development and demonstration at pilot scale will be combined with capacity building of local and regional actors. Solutions offered by VicInAqua are focussed on robustness, energy efficiency and economic viability in order to be adapted to the local challenges and to achieve high acceptance in peri-urban areas, where the sanitation infrastructure are poor and the demand for water is high.

The VicInAqua system is primarily designed to use for fish cultivation as here high quality water use is essential. For the pilot system, a Tilapia hatchery using RAS technology will be designed and constructed. RAS conserve water and reduce waste discharges. This hatchery will produce high quality fingerlings to supply pond aquaculture in the area. The RAS will be tailored to the local conditions and the output of the self-cleaning membrane bioreactor. The pilot will ultimately be up-scaled for adoption by aquaculture operators around the lake.





## **THE INTERNATIONAL AQUACULTURE FEED FORMULATION DATABASE (IAFFD): A STANDARDIZED, PUBLICLY AVAILABLE AQUACULTURE FEED FORMULATION DATABASE**

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The United States Soybean Export Council (USSEC) has worked for over 30 years with the aquaculture industry worldwide to promote a profitable, responsible, feed-based aquaculture industry while specifically promoting the use of United States soy products in aquaculture feeds. One of the primary targets for USSEC is the aquaculture feedmill industry, as this is where soy enters aquaculture production value chain. USSEC identified a critical weakness in the aquaculture industry that generally does not exist in the terrestrial livestock industry, the availability of a standardized feed formulation database. While a database is only a tool – an experienced formulator is needed to create realistic formulations – it is an important tool that was missing from the aquaculture industry.

Starting in late 2014 USSEC, in cooperation with other organizations, created the first known standardized aquaculture feed formulation database, initially named the Asian Aquaculture Feed Formulation Database (or AAFFD, due to an initial focus on the Asian aquaculture market). The feed ingredient sub-database contained information on over 200 feed ingredients and the species nutritional specification sub-database had information on 24 species at different age groups (typically six for fish and four for crustaceans). Since that time additional species and ingredients have been added, and the Stage III version expected in June 2017 should have at least 28 species and well over 400 ingredients, including non-Asian species, which is why it is now named the International Aquaculture Feed Formulation Database, or IAFFD.

This database is being made available to the public, at no cost, to help improve aquaculture feed formulation approaches. It is envisioned that this database could be used as an actual database, or could be used as a metric for comparison to an existing private database. It has also been used to create training workshops for aquaculture formulators, something that was difficult to do on a group basis without a common database to work from.

The database is available for download from the internet ([www.iaffd.com](http://www.iaffd.com)) and has already been imported successfully into several commercial feed formulation programs. This database concept is expected to be continually supported with modifications, additions, and improvements in the coming years.

## DEVELOPMENT OF DIGESTIVE ENZYMES AND UTILIZATION OF LOCAL FEED INGREDIENTS FOR VICTORIA LABEO (*Labeo Victorianus*) CULTURE

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The success of aquaculture industry depends on successful larval production and partly on reduction of fishmeal use in aqua feed because of its increasing demand, unstable supply and high price. The aim of this study was to describe the ontogeny and development of proteolytic enzymes and the influence of live feed during early larval ontogeny; evaluate weaning strategies for improved larval performance; and to determine the potential alternative protein sources for the formulation of least-cost optimal diets to satisfy the nutrient requirements of *Labeo victorianus* diets. Larval ontogeny was determined through enzymatic assays from day 1 to day 30 after hatching in larvae fed the following feed sequence: Rotifers during 3-15 day after hatching (DAH) and co-feeding between rotifer with formulated diet during 16-30. Three samples of larvae were collected before morning feeding on days 1, 3, 5, 7, 9, 12, 15, 20, 25 and 30 for enzymatic assays. Significant increases of all enzymes except acid phosphatase and chitinase were detected during 15-25 DAH ( $p < 0.05$ ) which was related to an increased growth of fish larvae at 30 DAH onward. Live feed, rotifers significantly increased enzyme activity of acid amylase and alkaline amylase of fish larvae during 3-15 DAH in comparison with those of unfed fish larvae ( $p < 0.05$ ). Larvae weaned using 50% rotifer and 50% formulated diet after 6 and 8 days post hatching resulted in significantly higher growth rates, Food Gain Ratio (FGR), Protein Efficiency Ratio (PER) and Productive Protein Values (PPV) than other treatments ( $p < 0.05$ ). The lowest growth performance was recorded in larvae weaned using 50% rotifer and 50% formulated diet immediately after the onset of exogenous feeding (3 day post hatch). The weight and length of the fish fed alternative protein sources measured weekly indicate that partial replacement of fishmeal with plant protein and rendered animal protein did not result in a significant decrease in specific growth rate (SGR) in *L. victorianus* fry during the first four weeks of the trial but there was a tendency of reduced growth. The present observations suggest that it is possible to substitute high quality fishmeal with different protein in the feed for *L. victorianus* fry.

## **POST-GRADUATE TEACHING AND CURRICULUM DEVELOPMENT IN AQUACULTURE AND FISHERIES SCIENCE IN SUB-SAHARA AFRICA: DEMAND, PRESENT OPPORTUNITIES AND FUTURE POTENTIAL**

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Sample of African Countries (Malawi, Egypt, Ghana, Uganda, Kenya, Madagascar, Mozambique and Zambia) are used to demonstrate inadequate postgraduate training opportunities in Africa. Postgraduate training needs in African Fisheries and Aquaculture has however been identified in the fields of catch assessment, stock assessment, environmental monitoring, ecosystem approaches, socio-economics, Geographical Information Systems (GIS), modelling, management and inland fisheries among others. Specific post-graduate training is intended to address the issues of inappropriate technologies and approaches, fish seed, unavailability of feed, and information management systems. As an example, Lilongwe University of Agriculture and Natural Resource Management (LUANAR), Malawi and the Malawi College of Fisheries (Department of Fisheries) offer certificates and degrees in aquaculture. In Egypt, there are a large number of Government research institutions and universities involved in postgraduate curriculum development in aquaculture and fisheries (e.g. the Universities of Cairo, Ein Shams, Alexandria, Suez Canal, El Azhar, El Mansura, Tanta, Asuit, Zagazig and Upper Egypt) specializing in postgraduate fisheries research and education subjects. The main postgraduate training institutions in Ghana include Renewable Natural Resources (Kumasi National University of Science and Technology - KNUST), Department of Fisheries and Oceanography (University of Ghana - UG) and Department of Fisheries and Aquatic Sciences (University of Cape Coast - UCC) that offer Ph.D, M.Sc and B.Sc programmes covering aquaculture nutrition, freshwater and brakish water aquaculture, fisheries, fish biology and conservation biology. Postgraduate work and postgraduate degrees are offered by the Zoology Department at the Faculty of Science and the Department of Wildlife at the Veterinary Faculty in Makerere University of Kampala (Uganda). Kenya offers Ph.D programmes in both Aquaculture and Fisheries Management at the University of Eldoret while similar programmes can be found in Uganda, Madagascar, Mozambique and Zambia. The highest postgraduate training potential and demand in many African countries revolve around fish genetics, selective breeding, fish nutrition, fish value chains, fisheries modelling and socio-economics. The curriculum development in many of these countries is constrained by inadequate expertise in curriculum development and limited funding opportunities. There seem to be inadequate training facilities, uncertain job opportunities and inadequate economic growth in the sub-sector. This review provides a list of training programmes and evaluates the current and potential impacts of these training on aquaculture development in selected countries in Africa. The review discusses the policy direction in achieving the postgraduate training and highlights some of the existing interventions in Africa.

## DEVELOPMENT OF LOW COST AQUAPONIC SYSTEM IN KENYA

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The University of Eldoret activities involved the design and testing of a low-cost aquaponic system that can be used for training, extension and by small-scale fish hobbyists in water deficient situations and urban/semi urban areas where land is scarce. An aquaponics prototype was developed and its efficiency assessed using different fish stocking densities. The system consists of a rectangular fish culture tank raising to 460 mm from the bottom and a plant bed raising to 270 mm from a raised platform, both units being arranged in a vertical tier. Water overflow from the fish unit is passed through a bio-filter made of cut plastic material to increase the surface area. This unit acts as a nitrification chamber before the water is pumped back to the plant unit using a submersible lift pump as shown in the appendices. Water discharge from the plant unit flows back to the fish unit by gravity thereby eliminating the need for double pumping. The prototype unit was tested using all male tilapia (*Oreochromis niloticus*) fry for 35 days to the fingerling stage. Results from the trial show that fish stocking density has an effect on the nutrient budget of the system. High nitrate content in the fish unit was associated with high stocking density of 80 Fish/T as compared to 60 Fish/T. The nitrification unit exhibited high efficiency since ammonia was not detected in the plant bed. Quantities of ammonia detected in the fish tanks after 35 days was close to the target values of zero. All important water quality parameters for aquaponics system such as DO, pH, alkalinity and Temperature were within optimum values, it is concluded that the system is viable and self regulating in terms of nitrogen cycle. The only limiting factor is the provision of other nutrients required for plant growth by supplemental fertilization. This report provides both design specifications and technical drawings of the aquaponics system developed during this activity. The unit offers good opportunities for rapid commercialization by the private entrepreneurs but there is need to improve on energy requirement through solar technology.

# THE EFFECT OF COMMERCIAL AND EXPERIMENTAL DIETS ON GROWTH PERFORMANCE OF *Oreochromis niloticus* L. FINGERLINGS REARED IN HAPAS IN UASIN GISHU COUNTY, KENYA

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Commercial diets are produced in bulk with significant amounts of anti-nutritional factors and toxic components increasing aquaculture production cost. Information about proximate composition of local feed ingredient is usually limited and unreliable. Unbalanced dietary amino acid contents increases de-amination and ammonia levels in water. This study intended to formulate on farm diets using locally available ingredients and balancing the Essential Amino Acids (EAAs) to enhance the physical quality and nutritive value for culture of *Oreochromis niloticus*. Four diets comprising methionine+lysine and lysine supplemented at 5.1 g kg<sup>-1</sup>, 2.7 g kg<sup>-1</sup> to non-EAAs supplemented and commercial tilapia diets at the University of Eldoret Fish Farm were tested. The diets consisted of 48% wheat bran, 30% freshwater shrimp, 18% cotton seed meal, 2% fish oil and 1% vitamin/mineral premix formulated at 30% crude protein before EAAs supplementation. The growth performance was conducted in hapas suspended in three earthen ponds (150 m<sup>2</sup>) each in a randomized design for 105 days. There were significant variations in temperature and pH, but still within suitable range for tilapia at 18-20°C and pH of 7.2 to 7.0. Dissolved oxygen over the period were at (4.8 to 6.2 mg L<sup>-1</sup>). The diets were estimated to provide about 17.17 MJ kg<sup>-1</sup> with about 22.9% digestible CP level, 8.03% ash and 90.7% dry matter. Diet 2 with lysine supplement exhibited better growth than other diets with a Phi prime (') of 3.441, Body Weight Gain of 289.8, Specific Growth Rate of 2.4, Food Conversion Ratio of 1.24 and Protein Efficiency Ratio of 2.68. This results show a high potential for on-farm fish feed formulation benefiting over 1000 fish farmers in formulating nutritionally balanced diets to improve growth and production of tilapia in Western Kenya. The protocol will be adopted to provide quality fingerlings at the at the University of Eldoret hatchery for fish farmers in Uasin Gishu County.

## ROLE OF SALINITY ON GROWTH PERFORMANCE OF *Oreochromis niloticus* AND *Oreochromis urolepis urolepis* HYBRIDS

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Influence of salinity on hybrids descended from *O. niloticus* ♀ and *O. urolepis urolepis* ♂ was investigated for 63 days. A total of 120 fry of  $0.29 \pm 0.01$ g were stocked in 1m<sup>3</sup> plastic tanks at a density of 10 fish / m<sup>3</sup> per tank. The experiment involved three salinity treatments 15, 25 and 35 with fresh water (2 salinity units) as control. The hybrids were fed on a balanced diet of 40% crude protein at 5% body weight twice a day. Water quality parameters were measured once a week. However, results on SGR, average weight gain and survival rate were not significantly affected by salinity ( $p > 0.05$ ). FCR differed significantly among treatments ( $p < 0.05$ ). The 25 Practical Salinity Unit (PSU) showed better growth performance than other treatments. Surprisingly all treatments showed better growth than control. When examined at the end of the study all hybrids were found to be 100% males. Length-weight relationship “b” values and condition factor “K” showed that hybrids had isometric growth, good health and were properly managed. It was concluded that if proper management is followed, the studied hybrids can be good candidates in both intensive and semi intensive mariculture by coastal people. The hybrids can solve the problem of stunted growth, be an alternative to the use of hormones in sex reversal.

## AN OVERVIEW OF MARKETS FOR FARMED FISH PRODUCTS IN SUB-SAHARAN AFRICA

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Africa's population is now just over one billion (and growing). This means more food for its people. Supplies of seafood in sub-Saharan Africa are still largely from wild fisheries, but the future of most of these fisheries is worrying. Aquaculture production though still relatively small in volume, has generated a tremendous growth in the last decade. In terms of demand, fish consumption per capita in sub-Saharan Africa currently stands at approximately 8.9 kg, compared to a world average of 19.7 kg and is projected to increase by 2.2 percent by 2025 (FAO), due to a number of factors.

The bulk of farmed fish in sub-Saharan Africa is destined for the region's domestic consumption, with low volumes of exports of high value products (shellfish, shrimps, seaweeds, ornamentals and other finfishes) to international markets. There has been a significant increase in seafood imports from outside sub Saharan Africa in the last decade.

Domestic market structures, systems and strategies for farmed fish products are diverse. These range from simple farm-gate sales to large, sophisticated distribution chains some of which produce processed products for export markets in developed countries. Women are increasingly becoming popular in the domestic distribution and marketing of freshwater fish products.

Farmed fish products are sold in various product forms ranging from primary products (live, fresh, chilled etc.) to secondary or value added products such as (smoked, dried, filleted, canned etc.). Wholesalers and retailers are increasingly becoming important channels for farmed seafood product marketing. There is a huge increase in the number of businesses that sell fish seed (live fingerlings or spat for the ever-growing grow-out farms in the region).

Besides improved aquaculture production volumes to satisfy demand, there are several other opportunities that lay ahead for sub-Saharan Africa to realise its full market potential for farmed fish products such as value addition, market diversification, product certification and standardisation.

Yet many countries of the region still face many challenges hampering the smooth operation of fish trade businesses. These include poor distribution infrastructures, insufficient policies to stimulate regional/international trade, lack of up to date marketing information, economic volatility of some African states and the high cost of doing business in many countries.





# METABOLIC EVALUATION OF FISH OIL REPLACEMENT BY *Plukenetia volubilis* OIL ON JUVENILES OF RAINBOW TROUT *Oncorhynchus mykiss*

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Marine fish oil (FO) is an excellent source of highly unsaturated fatty acids (HUFA) and essential in physiological processes, however, it is usually scarce. Rainbow trout has the ability to synthesize HUFA from essential fatty acids (EFA). *Plukenetia volubilis*, sacha inchi (SI) is an Amazonian plant, with high EFA content and balanced n3/n6 ratio. **Objective:** evaluate the effects of the substitution of FO by SI oil on the diet of *Oncorhynchus mykiss*, in the growth performance, energetic substrates, and in the enzymatic pathways involved in the synthesis of EFA.

**Materials and methods:** The experiment was conducted at Estação Experimental de Salmonicultura “Ascanio de Faria” - Campos do Jordão, SP. Three dietary treatments were evaluated (A, 100%OP; B, 50%OP: 50%SI; C, 100%SI). We used 90 juveniles of *Oncorhynchus mykiss* during 30 days, to analyze feeding behavior, acceptance of dietary treatments and growth performance. At the end, ten fish from each treatment were killed to collect the viscera to evaluate the somatic indices; muscle and liver were collect to evaluate the fatty acid (FA) profile and the gene expression of the enzymes involved in the (FA) synthesis pathway.

**Results:** No mortalities were recorded and fish responded well to the diets. The growth performance showed no significant difference (Table 1) among treatments. The muscle and liver FA profile are represented in tables 2 and 3. The enzymes involved in HUFA biosynthesis in the animals fed with 100% SI diet presented the highest gene expression levels, whereas the diet 50% OP-50% SI was intermediate for the elongases.

**Final considerations:** The SI oil has been well accepted and it did not change growth performance. Although the FA profile of muscle of the animals fed with 100%SI did not show high HUFA profile, the short period analyzed did not allow elongation and desaturation of PUFAs in HUFAs, however, molecular analysis allowed to suggest that in long term we can have better results with SI oil.

**Acknowledgement:** FAPESP (2015/23105-8).

**Table 1. Growth performance for rainbow trout**

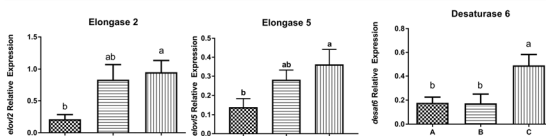
|                           | A            | B            | C            |
|---------------------------|--------------|--------------|--------------|
| Initial body weight (g)   | 92.49 ± 1.45 | 92.22 ± 1.45 | 91.91 ± 1.45 |
| Weight gain (%)           | 39.97 ± 3.82 | 36.49 ± 3.11 | 47.02 ± 3.99 |
| Specific growth rate (%)  | 1.17 ± 0.1   | 1.09 ± 0.08  | 1.34 ± 0.1   |
| Hepatosomatic indice (%)  | 2.93 ± 0.31  | 2.76 ± 0.21  | 2.94 ± 0.3   |
| Viceriosomatic indice (%) | 13.39 ± 0.69 | 11.45 ± 0.6  | 13.72 ± 0.81 |

**Table 2. Fatty acid composition of liver (%)**

|          | A              | B              | C              |
|----------|----------------|----------------|----------------|
| EPA      | 3.24 ± 0.26    | 3.07 ± 0.41    | 3.55 ± 0.41    |
| DHA      | 21.48 ± 1.91   | 22.8 ± 1.21    | 19.53 ± 1.69   |
| SFA      | 27.56 ± 1.02   | 25.28 ± 0.99   | 25.83 ± 1.08   |
| MUFA     | 41.16 ± 2.43 a | 27.96 ± 2.25 b | 23.7 ± 2.4 b   |
| PUFA     | 2.32 ± 0.26 c  | 12.3 ± 0.47 b  | 17.46 ± 0.83 a |
| PUFA n-3 | 0.48 ± 0.06 c  | 4.47 ± 0.15 b  | 6.5 ± 0.38 a   |
| PUFA n-6 | 1.91 ± 0.23 c  | 7.83 ± 0.43 b  | 10.96 ± 0.54 a |
| HUFA     | 31.07 ± 2.13   | 34.46 ± 2.27   | 32.81 ± 1.62   |
| HUFA n-3 | 26.69 ± 2.29   | 28.35 ± 1.89   | 26.18 ± 1.72   |
| HUFA n-6 | 2.58 ± 0.24 b  | 6.11 ± 0.54 a  | 6.64 ± 0.35 a  |
| n-3/ n-6 | 6.15 ± 0.53 a  | 2.37 ± 0.13 b  | 1.89 ± 0.13 b  |

**Table 3. Fatty acid composition of muscle (%)**

|          | A              | B              | C              |
|----------|----------------|----------------|----------------|
| EPA      | 7.18 ± 0.37 a  | 4.86 ± 0.35 b  | 1.87 ± 0.17 c  |
| DHA      | 20.65 ± 0.93 a | 12.53 ± 1.22 b | 8.64 ± 0.83 c  |
| SFA      | 29.87 ± 0.27 a | 26.49 ± 0.53 b | 24.78 ± 0.78 c |
| MUFA     | 28.7 ± 1.04    | 29.34 ± 1.51   | 30.77 ± 1.47   |
| PUFA     | 8.57 ± 0.41 c  | 20.33 ± 1.09 b | 29.56 ± 2.16 a |
| PUFA n-3 | 1.69 ± 0.13 c  | 8.28 ± 0.65 b  | 12.99 ± 1.48 a |
| PUFA n-6 | 6.88 ± 0.39 c  | 12.05 ± 0.54 b | 16.57 ± 0.73 a |
| HUFA     | 32.86 ± 1.15 a | 21.47 ± 1.54 b | 14.9 ± 1.19 c  |
| HUFA n-3 | 30.12 ± 1.12 a | 19.02 ± 1.45 b | 12.03 ± 1.07 c |
| HUFA n-6 | 2.74 ± 0.16    | 2.46 ± 0.18    | 2.87 ± 0.14    |
| n-3/ n-6 | 3.39 ± 0.24 a  | 1.92 ± 0.14 b  | 1.29 ± 0.06 b  |



**Figure 1.** Relative gene expression of hepatic enzymes involved in FA synthesis pathway.

## THE USE OF MOLECULAR BIOLOGY TOOLS TO IMPROVE AQUACULTURE PRODUCTIVITY: THE EPPO EXPERIENCE

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The Aquaculture Research Center of Olhão (EPPO) from the National Institute of Ocean and Atmosphere (IPMA) stands out for the unique experimental conditions on aquaculture at the national level. EPPO facilities allows from the most fundamental research to pilot studies that can help to give answers to the aquaculture sector. In analytic terms the EPPO has well equipped laboratories to perform a number of techniques, such as biochemistry, microbiology and molecular biology, among others. In recent years the advance on molecular biology of aquatic animals has provided useful information that can be used to improve aquaculture and the welfare conditions of farmed fish.

Some of the techniques that have been developed in the EPPO facilities during the last few years include:

- The parental contribution of several breeder species: molecular markers (e.g. microsatellite sequences) present in genomic DNA extracted from breeders and offspring samples are analyzed and compared in order to infer the parental contribution of progenitors;
- Identification of molecular biomarkers to evaluate welfare conditions of farmed fish: molecular biomarkers are identified combining literature search and transcriptomic-based approaches. Selected molecules are validated for their potential to infer on welfare conditions of farmed-fish;
- Gene expression analysis of growth-related genes: gene expression of growth-related genes is evaluated in RNA extracted from fish grown in different farming conditions (e.g. with different nutritional requirements) and in selected time periods. The figure presented below shows the preliminary results of one long-term experience performed in EPPO facilities where the insulin growth factor 1 (IGF1) gene expression was evaluated at 350 days after hatching (DAH).

A tendency for an increase of IGF1 expression in RP and for a decrease in 38HP treatment was observed at 350 DAE and was in agreement with growth data collected, suggesting that this kind of approach is an interesting and useful tool to evaluate the quality and performance of fish growth.

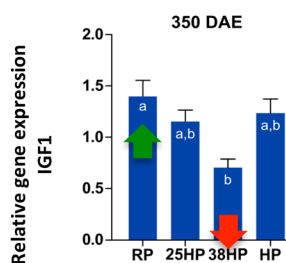


Figure 1 – Relative gene expression of IGF1 at 350 DAH in fish fed with different diets, containing different protein content (RP, 25HP, 38HP and HP).

Acknowledgments: The authors thank to J. M. Mancera and K. Mohammed-Geba for supplying the transcript sequences used in the study. The research was funded by DIVERSIAQUA project (MAR2020, Portugal).

## MODELS FOR INCLUSIVE AQUACULTURE DEVELOPMENT IN AFRICA

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Africa's population is expected to double from its current 1.2 billion people to 2.4 billion in 2050. If fish consumption in Africa stays at the same level, fish production (or importation) needs to increase with almost 12 Million tonnes. Most capture fisheries are either at their maximum, or over-fished, therefore, if Africa wants to produce its own fish, aquaculture would be the only alternative.

Total fisheries production in Africa is 10 515 842 tonnes (2015), of which approximately 18% comes from aquaculture (1 861 271 tonnes in 2015). The biggest producers are:

- Egypt (1 137 091 tonnes mainly tilapia, cyprinids and mullets),
- Nigeria (313 000 tonnes, mainly African catfish and Tilapia),
- Zanzibar (133 000 tonnes, mainly *Eucheuma denticulatum*, a red algae), and
- Uganda (111 023 tonnes, mainly African catfish and tilapia).

There is a vast potential for aquaculture to grow in Africa. At present this potential for aquaculture remains largely untapped, only Egypt has been able to reach the scale of production achieved in other parts of the world. In 2005, during the Fish for All summit in Abuja, the NEPAD Action Plan for the Development of African Fisheries and Aquaculture was formulated, and forms the core of FAO's approach assisting countries with the development of the aquaculture sector in Africa. Key to the NEPAD Action Plan are the development of national strategies, zoning/clustering of farms, and support to small and medium enterprises. FAO has assisted several countries with the formulation of their aquaculture sector development strategies, has produced (together with the World Bank) policy guidance with respect to aquaculture zoning, and assisted countries with the development of projects implementing national aquaculture development strategies.

The clustering of aquaculture enterprises has several advantages which will be crucial for the development of an aquaculture sector. These clusters will be beneficial for groups of farmers seeking joint access to feed, seed, technical support services, markets and post-harvest services.

During the presentation several models of aquaculture enterprise clustering will be discussed.

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## PERFORMANCE OF WHITE LEG SHRIMP (*Litopenaeus vannamei*) FED HIGH OR LOW FISH MEAL DIET SUPPLEMENTED WITH INCREASING LEVELS OF DL-METHIONYL-DL-METHIONINE

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Shrimp feed industry is constantly looking for opportunities to reduce inclusion of dietary fish meal. Replacement of fish meal with alternative protein sources requires balancing diets for dietary nutrients, while maintaining palatability. Methionine (Met) is typically the first limiting amino acid in soybean-based shrimp feed and thus, supplementation of methionine is essential in those diets. DL-Methionyl-DL-Methionine, 'Met-Met' in short (AQUAVI® Met-Met), is a dipeptide of DL-Methionine and is shown to be an effective supplemental methionine source for shrimp in the recent studies. The objective of the current study was to evaluate and compare the performance of shrimp fed low fish meal diet (8%) with increasing levels of supplemental Met-Met, relative to those fed a high fish meal diet (16%) under green water system.

Shrimp (3.47±0.02 g, mean ± SD, initial weight) were randomly stocked into 20 cages (1×1×1.5 m). The experiment consisted of five dietary treatments (~38% crude protein) including: a positive control (PC) diet containing 16% fish meal with 0.10% DL-Met supplementation (D1), a negative control (NC) diet containing 8% fish meal without supplemental methionine (D2) and three other NCs with increasing levels of Met-Met supplementation at 0.03%, 0.06%, and 0.10%, respectively (D3-D5). Analyzed Lys, Met and Met+Cys levels (as-is basis) were 1.83%, 0.76% and 1.23%, respectively in the PC diet and 1.75-1.81%, 0.57-0.72% and 1.05-1.20%, in the NC diets with or without supplemental Met. Each dietary treatment was allotted randomly to four replicate cages and shrimp were fed four times daily to apparent satiation for 42 days. Data were analyzed with ANOVA and means were separated using Tukey test ( $P < 0.05$ ).

Results of the study are shown in Table 1. Shrimp fed the NC diet showed significantly lower weight gain and feed intake, marginally lower specific growth rate (SGR) ( $P=0.06$ ) and similar feed conversion ratio (FCR) relative to those fed the PC diet. Growth performance and FCR significantly improved for the groups fed with increasing levels of supplemental Met-Met (D3-D5). Shrimp fed NC+0.10% Met-Met diet (0.72% Met and 1.20% Met+Cys) showed the best performance with significantly better body weight gain, and FCR relative to the group fed NC diet without supplemental Met. Overall, results showed that AQUAVI® Met-Met can be used as an effective supplemental methionine source in reducing fish meal level from 16% to 8% in shrimp feed without compromising growth performances.

**Table 1. Growth performance (mean ± SD) of shrimp fed high or low fish meal diets supplemented**

| Diets      | Weight gain<br>(g/shrimp) | SGR<br>(%/d)   | Feed Intake<br>(g/shrimp) | FCR<br>(g fed/g biomass gain) | Survival<br>(%) |
|------------|---------------------------|----------------|---------------------------|-------------------------------|-----------------|
| PC         | 8.60 ± 0.26bc             | 2.95 ± 0.06abc | 12.51 ± 0.18b             | 1.72 ± 0.08ab                 | 84.00± 0.82     |
| NC         | 7.58 ± 0.71a              | 2.75 ± 0.15a   | 11.48 ± 0.53a             | 2.05 ± 0.13a                  | 77.25± 2.50     |
| NC+0.03%MM | 8.26 ± 0.42ab             | 2.89 ± 0.10ab  | 12.28 ± 0.31b             | 1.75 ± 0.07ab                 | 84.00± 1.83     |
| NC+0.06%MM | 9.02 ± 0.04bc             | 3.04 ± 0.03bc  | 12.13 ± 0.12ab            | 1.58± 0.05ab                  | 84.67± 2.08     |
| NC+0.10%MM | 9.36 ± 0.32c              | 3.11 ± 0.09c   | 12.10 ± 0.19ab            | 1.49± 0.07b                   | 85.75± 1.71     |

**with increasing levels of Met-Met over 42 days**

PC, positive control; NC, negative control; MM, DL-Methionyl-DL-Methionine (Met-Met)  
Means in each column sharing different alphabets are significantly different ( $P < 0.05$ , ANOVA)

## PROGRESS IN THE AMINOACID NUTRITION OF NILE TILAPIA

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Rising feed cost is a major challenge for tilapia producers. Protein is the most expensive nutrient in the diet and there has been continuous efforts in finding alternative cost-effective protein sources for tilapia. Quality of the dietary protein is determined by its amino acid composition and digestibility in relation to the requirements of fish. Therefore, it is very critical to improve our understandings on the amino acid requirements of tilapia in order to be flexible with the diet formulations. Knowledge on the amino acid requirements of tilapia is increasing over the years. Quantitative requirements for essential amino acids (EAA) of Nile tilapia was first reported back in 1980s. Since then several studies ( $n > 15$ ) have been published on the amino acid requirements of Nile tilapia. In due course, efforts were also made to provide amino acid recommendations for feed industry which included table recommendations given by NRC, and also by Evonik for different growth stages of tilapia. The main objective of this paper is to do a critical and comprehensive review of the available datasets and translate the results into recommendations.

Studies in the past have used dose-response approach, diet dilution technique and deletion method for determining amino acid requirements of tilapia, with dose-response approach being the most commonly used method. Among the EAA, requirements for sulfur amino acids (methionine and cysteine), lysine and threonine were most commonly studied, understandably because these are often the first three limiting amino acids in practical fish feeds. Table 1 illustrates the summary of various published data on the amino acid requirements of Nile tilapia. We find a wide range of variations in the requirement data published for several EAA. For example, dietary requirements (% diet, total basis) varied from 0.49 to 1.13% for methionine, 0.85 to 1.46% for methionine + cysteine, 1.43 to 2.32% for lysine, 0.83 to 1.45% for threonine, 1.18 to 1.95% for arginine and 0.48 to 0.82% for histidine. Few recent studies ( $n=3$ ) showed a dietary requirement of  $1.81 \pm 0.32\%$  (mean  $\pm$  SD) for lysine on digestible basis. Variations among studies are partly because of differences in methods used and mathematical models adopted for the estimation of EAA requirements. Furthermore, Nile tilapia has been genetically improved over the years for better growth which suggests that dietary EAA requirements for modern tilapia can be quite different and likely higher than the previously reported values. In the requirement studies, one of the basic assumptions to be met is that treatment diets sufficiently meet requirements of all other EAAs except the EAA under study. Violating this assumption can lead to underestimation of amino acid requirements which may partly explain lower requirement data published in some studies. Finally, this paper also attempts to provide EAA recommendations for different life stages and production scenarios of Nile tilapia based on factorial approach using available data on maintenance requirements, amino acid retention and utilization.

**Table 1. Summary of essential amino acid (EAA) requirements (% diet, total basis) of juvenile Nile tilapia from published studies**

<sup>1</sup> = number of studies used; <sup>2</sup> = standard deviation

| EAA             | Met         | Met+Cys     | Lys         | Thr         | Trp         | Arg         | Iso         | Leu         | Val         | His         | Phe+Tyr     |
|-----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| N <sup>1</sup>  | 7           | 7           | 5           | 5           | 2           | 3           | 2           | 2           | 2           | 3           | 2           |
| Mean (% diet)   | <b>0.82</b> | <b>1.11</b> | <b>1.75</b> | <b>1.17</b> | <b>0.33</b> | <b>1.55</b> | <b>0.88</b> | <b>1.23</b> | <b>0.97</b> | <b>0.61</b> | <b>1.56</b> |
| SD <sup>2</sup> | 0.21        | 0.26        | 0.35        | 0.24        | 0.06        | 0.39        | 0.01        | 0.39        | 0.26        | 0.18        | 0.01        |

## **HARNESSING ADVANCES IN MOLECULAR GENETICS FOR AQUACULTURE AND FISHERIES TRAINING IN AFRICA**

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Africa's population is on a rapid increase and will hit the 2 billion mark in 2050. This population needs to be fed, by food being produced from the finite land and water resources. There are increasing trends that favour the consumption of fish. However the capture fisheries can no longer sustain the supply of fish. This therefore calls for establishing and strengthening aquaculture enterprises across Africa. These enterprises need qualified and fit for purpose staff at all levels. This is where the Higher Education institutions need to play a major role in designing and implementing appropriate and relevant training courses for the graduate products. The molecular genetics field has seen rapid advances in the recent years. These advances span a wide field including but not limited to population genetics, selective breeding, molecular markers for traceability, aquatic disease diagnostics and epidemiology, and identification of genetic loci for desirable loci. In an effort to keep with the pace of the changing landscape in aquaculture, it is high time African education institutions teamed up with research institutions with a view of improving the teaching curricula. This approach will bring the desired cutting research in molecular genetics and tailor it for use in aquaculture training for improved production and supply of fish to the ever-growing population.



## CHALLENGES FACING A RURAL ENTREPRENEURIAL FARMER IN PLANNING AND DEVELOPING AN AQUAPONICS FACILITY IN SOUTH AFRICA: A CASE STUDY FROM THE EASTERN CAPE

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Freshwater aquaculture is considered the oldest fish farming sector in South Africa that, however small in comparison to global trends showed significant increase in production volumes the past two decades. As the current majority of freshwater finfish aquaculture is commercially produced, there is much opportunity for individuals from rural communities to become involved in subsistence and/or artisanal level aquaculture.

Several small, medium and micro-sized enterprises (SMMEs) wanting to invest in aquaculture have been identified by the National Department of Agriculture, Forestry and Fisheries (DAFF) to stimulate socio-economic growth in support of job creation and food security. However, the majority of these rural SMMEs do not have the financial means or skilled capabilities to comply with the numerous environmental authorisations governing the aquaculture sector in South Africa.

In 2014, the National Department of Environmental Affairs (DEA) has initiated the Special Needs and Skills Development (SNSD) programme, currently managed by the Council of Scientific and Industrial Research (CSIR). The programme aims to assist rural SMMEs by undertaking a Basic Assessment to obtain Environmental Authorisation (EA) to develop and legally operate an aquaculture facility.

This paper presents a case study from the Eastern Cape Province of South Africa, wherein the SNSD programme is assisting a community-based enterprise to obtain EA for the development of an aquaponics facility near Coffee Bay. This enterprise known as Intubayethu Aquaponics plans to establish an aquaponics facility producing vegetables and Nile tilapia in a self-contained system.

Challenges faced by this rural entrepreneur in obtaining their EA include *inter alia* the inability to produce a detailed project description, an adequate business plan and detailed technical designs of their proposed aquaponics system. Further complications arose from lack of sufficient funding, limited knowledge of the regulatory environmental requirements pertaining to aquaculture, and landowner conflict with the Ntubeni Tribal Authority. In addition to these challenges, the proposed facility is situated in a protected area which may not be deemed feasible by the authority. Based on the sensitivity of the proposed site, an alternative site will be included and assessed in the Basic Assessment. This paper will provide details on the process followed and the outcome thereof.

## OPTIMIZING SOME BREEDING FACTORS OF CULTURED NILE TILAPIA *Oreochromis niloticus*, UNDER BRACKISH WATER

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Tilapia is one of the most important cultured fish in the world. World production of tilapia is highly increased in recent decades. Studies about tilapia in Iran have begun from 2008. In the present study, sex ratio, stocking density, photoperiod, water salinity and replacement period of female brooders of the Nile tilapia, *Oreochromis niloticus* were investigated.

Tilapia is one of the most important cultured fish in the world. In 2012 the global volume of farmed fish exceeded global volume of beef for the first time (Fitzimmons et al., 2014). Nile tilapia, *O. niloticus* is the most common tilapia widely cultured in many tropical and subtropical countries and consists up to 80% of cultured tilapias of the world (FAO, 2013).

Tilapias can live in different aquatic ecosystems due to their reproductive strategies. On the other hand, the gap between seed supplies and farmers' demand is one of the most serious limitations for tilapia culturists. Notice about different effective agents on tilapia reproduction aspects might be useful for increasing of production and better management of tilapia hatcheries.

Studies about tilapia were started in Iran from November 2008. The reproductive aspects of tilapias including absolute fecundity, spawning intervals and hatch percents of cultured *O. niloticus* at brackish water condition were studied previously.

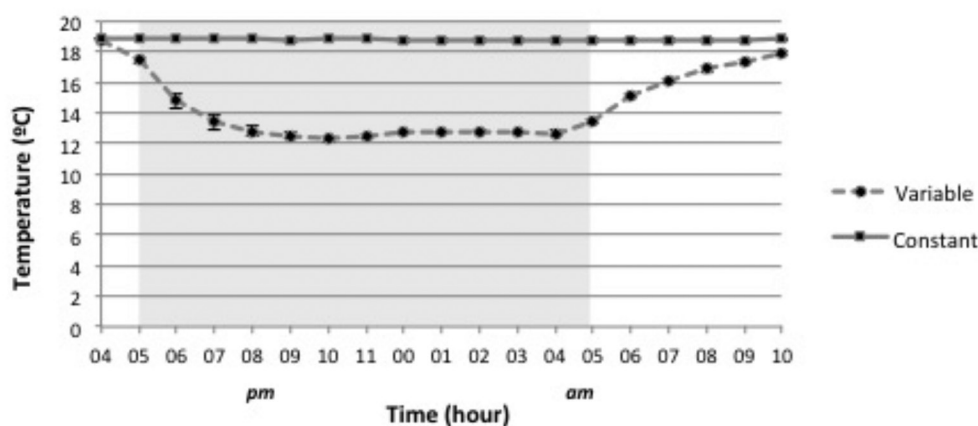
In the present study, reproduction indices were surveyed to show optimums of sex ratio, stocking density and photoperiod, water salinity and replacement periods of brooders, in the present study.

## GILTHEAD SEABREAM *Sparus aurata* PERFORMANCE EXPOSED TO LONG-TERM DAY/NIGHT TEMPERATURE VARIATIONS

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Fish growth is normally enhanced at higher temperatures. Still, heating water to enhance fish growth, thus reducing fish production cycle, is extremely expensive compromising firm viability. Solar energy technologies may provide affordable energetic solutions to bypass this obstacle. Since with solar energy, water is heated only during the day period, what would be the impact for fish overall performance? The aim of this study was to compare gilthead seabream (*Sparus aurata*) performance when reared at two thermic scenarios (Figure), constant water temperature (20°C) and daily fluctuations of water temperature (20°C during day and 13°C at night). Trial ended after 60 days, when fish samples were collected to evaluate fish overall performance. Fish growth at constant water temperature was 37,7% higher than the variable thermic scenario. Additionally the feed conversion rate (FCR) was also 11,3% more efficient. Despite better growth parameters, fish cultivated to constant water temperature required higher costs than the fish exposed to water temperature daily fluctuations. The food and oxygen consumptions were respectively 27.5% and 46.4% higher than the fish cultivated at water temperature variations. Fish welfare was also assessed by other analytical parameters (haematological, plasmatic and biochemical), which will also be presented.



**Figure - Daily temperature water profile of the thermic scenarios studied (constant and variable).** The shaded area represents the water-cooling period (5pm to 5am).

## HELMINTH PARASITES OF TILAPIA (*Oreochromis Niloticus*) IN SMALL WATER BODIES IN UASIN GISHU AND SIAYA COUNTIES, KENYA

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For sustainable development of culture-based fisheries (CBF) development in small water bodies (SWBs), establishment of effects of parasites on cultured fish should be established. Studies were conducted from November 2010 to July 2012 in Kesses and Kerita dams in Uasin Gishu and Mauna and Yenga dams in Siaya to determine the effect of rainfall seasonality and sex of fish on helminth parasites of tilapia (*Oreochromis niloticus*). Fish were collected from the small water bodies once a month for twelve months. Parasitological examination was done according to standard procedures. In Kesses dam, fish infected with *Tylodelphys sp.* had a significantly higher condition factor than un-infected ones during the dry season. Enhancement of fisheries production in SWBs should take into consideration the role seasonal changes on parasite dynamics and their effects on fish hosts. Furthermore, there is also need to create awareness on the risk of transmission of some fish parasites to consumers.

## **THE ROLE OF MOBILE PHONES IN FACILITATING AQUACULTURE DEVELOPMENT IN UGANDA**

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Aquaculture is growing fast globally but challenged by inadequate information and technical assistance to guide farmers in developing countries. Use of mobile phones could improve aquaculture productivity by increasing access to technical guidance, extension services, product assembly, input coordination, and price discovery for small and medium-scale fish farmers. However, little is known about the potential impact of mobile phone use on aquaculture development and conveying needs and interests of fish farmers in Uganda.

This study sought to (1) identify the needs and interests of fish farmers on the use of mobile phones; (2) identify the potential benefits of the use of mobile phones among fish farmers; (3) examine the reported experiences and perception of mobile phone use among fish farmers; and (4) examine the main obstacles for adoption of mobile phones by fish farmers. Qualitative data was generated through five focused group discussions with fish farmers which were conducted in Masaka, Bushenyi, Mpigi, Kalungu and Mukono districts of Uganda. Thematic content analysis was used to analyze the data.

The main findings reveal that fish farmers use mobile phones to access technical guidance from intermediary farmers, obtain market information, accomplish mobile banking, contact family members and make plans for procurement of fish farming inputs. Factors influencing mobile phone use included lack of electricity, poor network coverage, high calling credit and maintenance costs, lack of awareness and promotion. Information regarding site selection, pond construction, stocking, harvesting, feeding, sources of quality feeds and fingerlings, diseases and predator management, water quality management, sampling, and price discovery were most needed by fish farmers. Development of a cell-phone based fish application can thus enable fish farmers access necessary agricultural information and current market prices in order to improve productivity and profitability.

## **DESIGN OF MOBILE PHONE APPLICATION TO FACILITATE INFORMATION SHARING AMONG FISH FARMERS IN UGANDA**

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Mobile phone technology play a vital role in enhancement of fish farming business worldwide. This study aims at implementing the development of a mobile phone application (aquaculture App) that will enable fish farmers in Uganda access fish farming, marketing, and input discovery information at their convenience. The objectives include (1) assessing the socio-economic characteristics of the fish farmers (2) examining the critical information needs of fish farmers that will be used in the development of a mobile application service that will enable fish farmers, input suppliers, fish traders, and fish farmer's association leaders access fish farming and market information.

Snowball sampling techniques will be used to identify participants in Mpigi, Mukono and Wakiso districts of Uganda. Intensive interviews (focused group discussions and face to face interviews) will be used during data collection. An interview guide with open ended questions will be used to generate both qualitative and quantitative data. A specialized software (Atlas-ti) to sort information obtained through these conversations will be employed. The findings of this study will guide Public agencies, non-governmental organizations, researchers and cellular service providers in facilitating the use of cell phone as a means to guide, coordinate, and instruct fish farmers in Uganda.

## SOCIO-ECOLOGICAL SUITABILITY FOR FISH PRODUCTION IN SMALL WATER BODIES IN SOUTH WESTERN UGANDA, EAST AFRICA

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The worldwide increase in fish production is largely due to utilization of natural water bodies for aquaculture but fish restocking has also played a major role. There are 160 small (10-350 km<sup>2</sup>) lakes in western Uganda where fish per capita consumption (4kg) is below the country's average of 8.3kg. The production potential of these lakes remain largely unknown. This study examined the suitability of fish production in five of the lakes: Mulehe, Chahafi, Kayumbu, Mutanda (Kisoro district) and Lake Bunyonyi (Kabale district), South Western Uganda by determining their water quality, biological and socio-economic conditions.

Water quality attributes: temperature (21.1–27.6°C), dissolved oxygen (5.2–8.8mgL<sup>-1</sup>), transparency (0.4–2.1m), total depth (5.7–60.8m), conductivity (131–261µScm<sup>-1</sup>), salinity (0.02–0.14ppt) and pH (6.2–8.0) exhibited wide ranges as did biological attributes namely phytoplankton bio-volume (317.2–2635.4mm<sup>3</sup>L<sup>-1</sup>), macro-invertebrate density (171–2498 individuals/m<sup>2</sup>) and fish (672–4598 individuals). Subsistence fishing was evident on all the lakes with 351 boats, employing 523 fishing crew using 643 gill nets (>70% below 101.6 mm mesh size (4")) as the main fishing inputs with annual harvested fish of 300 tones worth 2.8 Billion shillings (USD \$800,000).

Most physico-chemical and biological attributes fell within recommended ranges (Queensland Water Quality Guideline, 2009) for fish growth except temperature which was below but within the acceptable limits. Some identified threats to aquatic productivity were cultivation up to the lake margins, overfishing, predation and

parasite infestation. Restocking and aquaculture of two herbivorous fishes (*Oreochromis niloticus* and *Oreochromis esculentus*) are recommended for increasing fish production in the lakes, supported by effective management (gear and area restrictions), restoration of the buffer zone to the recommended 50m for small water bodies.

TABLE 1. Selected physicochemical parameters of the water from the five sampled lakes in South-western Uganda, indicating the optimum ranges for fish production in the last column.

|                                 | Bunyonyi | Chahafi | Kayumbu | Mulehe  | Mutanda | Optimum    |
|---------------------------------|----------|---------|---------|---------|---------|------------|
| Surface area (Km <sup>2</sup> ) | 61       | 1.0     | 2.2     | 4.1     | 26.4    |            |
| Depth (m)                       | 3.3-41.5 | 0.5-5.4 | 0.5-5.3 | 0.5-6.0 | 1.2-60  | >2 and <20 |
| Temp (°C)                       | 21.5     | 21.6    | 21.5    | 21.4    | 22.1    | 21-32      |
| DO (mg/L)                       | 6.4      | 6.8     | 6.1     | 8.2     | 8.4     | >3.0       |
| pH                              | 7.1      | 7.9     | 7.8     | 7.7     | 7.1     | 6.8-9.5    |
| Cond (µS/cm)                    | 236.6    | 218.6   | 142.7   | 230.7   | 247.4   | 100-2,000  |
| SD (m)                          | 1.6      | 0.6     | 0.6     | 0.5     | 2.0     | 0.5-0.6    |
| TP (mg/L)                       | 0.08     | 0.09    | 0.16    | 0.19    | 0.10    | <1.0       |
| NO <sub>3</sub> -N (mg/L)       | 0.06     | 0.06    | 0.07    | 0.09    | 0.06    | 0-1        |
| NO <sub>2</sub> -N (mg/L)       | 0.004    | 0.006   | 0.009   | 0.006   | 0.006   | <4         |
| Salinity (ppt)                  | 6.5      | 16.2    | 14.6    | 10.1    | 2.6     | 15-33      |
| TSS (mg/L)                      | 3.1      | 5.4     | 6.9     | 5.4     | 2.6     | >10        |

TABLE 1. The biological fish component of the five sampled lakes in South-western Uganda, an indication to support fish production'

| Fish taxa                    | Bunyonyi | Chahafi | Kayumbu | Mulehe | Mutanda |
|------------------------------|----------|---------|---------|--------|---------|
| Mosquito fish                | 1        | 1       | 1       | 0      | 1       |
| Haplochromines               | 3        | 4       | 5       | 4      | 4       |
| Tilapia                      | 1        | 2       | 1       | 2      | 2       |
| Clarias spp                  | 2        | 2       | 3       | 2      | 2       |
| Barbels spp                  | 2        | 1       | 1       | 1      | 1       |
| Cray fish                    | 1        | 1       | 1       | 0      | 1       |
| Total number of species      | 10       | 11      | 12      | 9      | 11      |
| Number of individuals        | 924      | 672     | 1593    | 4598   | 1706    |
| Density (individuals/sq. km) | 15       | 672     | 724     | 1121   | 65      |

TABLE 3. The biological algal component of the five sampled lakes in South-western Uganda, a base of fish food and therefore fish production

| Algal groups                                   | Bunyonyi | Chahafi | Kayumbu | Mulehe | Mutanda |
|--|----------|---------|---------|--------|---------|
| Blue Green                                     | 14       | 15      | 15      | 23     | 13      |
| Green algae                                    | 9        | 12      | 14      | 16     | 10      |
| Diatoms  | 7        | 3       | 4       | 8      | 8       |
| Dinoflagellates                                | 1        | 0       | 1       | 1      | 1       |
| Total Number of species                        | 31       | 30      | 34      | 48     | 32      |
| Bio-volume (mm <sup>3</sup> /L <sup>-1</sup> ) | 1356     | 389     | 312     | 2626   | 1460    |
| Bio-volume per Sq. Km                          | 22.2     | 388.5   | 141.9   | 640.6  | 55.3    |



## IMPROVED TECHNIQUE FOR HARVESTING MAGGOTS, USED AS START-UP DIET FOR *Clarias gariepinus* FRY

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The study was conducted with the aim of developing a least cost and balanced diet for raising *Clarias gariepinus* fry. Copra, was used to produce a nonconventional source of protein, maggots that were used in formulating diets that were administered to catfish fry. Maggots were cultured in copra substrate, and harvested using concentration cups of various sizes. Turn-up of maggots was monitored every 12 hours in both hollow cups and petri dishes while taking their total length and weight on both wet and dry basis. Mean total length of between 1.0-1.5cm and a mean of 0.01-0.021g wet weight was captured. Maggot production depended significantly on cup size, moisture content and quantity of substrate. Copra improved (CIM) and Moringa fortified (MFM) diets were formulated from the copra substrate with maggots. Performance of these diets were compared to performance of Fishmeal (FM) and Decapsulated Artemia (DA). Experiment was set in a complete randomized design. A 48day growth trial was conducted on a cohort of 168 fry obtained by artificial propagation with mean initial weight of 0.119g and total length of 0.92cm. The stocking density was 14fry per tank, set in an outdoor static system. Water quality was maintained within optimum levels. Sampling was done bi-weekly. Survivors were counted after each sampling. There was total mortality in tanks maintained on CIM diet on Day 30, which so far had the best performance in Specific Growth Rate (SGR) with a peak of 27%/day. Food conversion efficiency of upto 92.838% was recorded for MFM, which also had the best performance of 1.077 FCR, as opposed to FM (1.754) and DA (1.938). The best performance was recorded in MFM diets which had mean weight of 0.483g significantly higher than DA (0.056) and FM (0.045). Concentration cups is an efficient method of harvesting maggots. For better performance, maggot diets obtained from copra substrate should not be used in isolation.

## A PRELIMINARY INVESTIGATION INTO THE POTENTIAL EFFECT OF *Artemisia afra* ON GROWTH, SOME HAEMATOLOGY AND BIOCHEMICAL PARAMETERS AND DISEASE RESISTANCE IN SUB-ADULTS OF *Oreochromis mossambicus*

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The main objective of this study was to investigate the potential of an indigenous plant, *Artemisia afra*, on growth, haematology and biochemical and disease resistance in *Oreochromis mossambicus*. Five *A. afra*-based diets (0%, D1; 1%, D2; 2%, D3; 3%, D4; 4%, D5) were formulated and randomly fed to triplicate groups of 10 fish ( $32.5 \pm 1$  g). The fish were fed for 45 days in a recirculating system. Fish fed the control, 1%, and 2% *A. afra* inclusion showed significant ( $P < 0.05$ ) improvements in weight gain and specific growth rate. Feed conversion ratio increased with increasing levels of *A. afra*. No significant differences ( $P > 0.05$ ) were observed in RBCs (red blood cell counts) and HCT (haematocrit) across diets, but RBCs were higher in the control. Haemoglobin levels were significantly lower ( $P < 0.05$ ) only in D5 as compared to the control and D2-D4. No significant differences ( $P > 0.05$ ) were observed in MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin) and MCHC (Mean corpuscular haemoglobin concentration) across diets. The ALT (Alanine Transaminase), AST (Aspartate Transaminase) and ALP (Alkaline Phosphatase) levels showed no statistically significant difference ( $P > 0.05$ ) between the control and the *A. afra* inclusion levels. This suggests that the plant extract did not have a negative effect on haemato-biochemical parameters. There was an increase in Total Protein and Albumin with increases in *A. afra* inclusion levels. Phagocytosis and lysozyme activity were also higher ( $P < 0.05$ ) in fish fed *A. afra*-based diets than the control. Relative percentage survival and the survival rate of *O. mossambicus* were higher in fish fed D4 and D5, indicating that the use of *A. afra*-based diets enhances disease resistance in *O. mossambicus*. Furthermore, white blood cells were higher in fish fed the *A. afra*-based diets than in the control. This again indicates an improved defence system against pathogens.

## LOBSTER MOVEMENT AROUND MUSSEL FARMS IN EASTERN CANADA

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Suspended bivalve aquaculture is an important economic activity in many temperate coastal communities. As this industry grows, concerns about its interactions with the environment have increased. Suspended mussel culture may have a number of effects on the benthic environment, including increasing the abundance of commercially-important scavenging species within the confines of farm sites. In this study, we evaluated the use of mussel aquaculture sites by the American lobster *Homarus americanus*, one of the most important commercial species in eastern Canada.

Lobsters movements were recorded in and around three mussel farms (one off-shore of îles-de-la-Madeleine, Québec, and two in Malpeque Bay, Prince Edward Island, IDM, MB1, and MB2, respectively) on different occasions using a passive fine-scale acoustic telemetry positioning system (VEMCO, Figure 1). All 60 of the tagged lobsters left the 14 km<sup>2</sup> study area within 24 hours; those within the farm left the area in random directions whereas those outside of the farm left in the NE direction. Lobsters that returned to the farm spent more time there and concentrated their activities in smaller areas than lobsters that returned to areas outside of the farm. Lobsters in MB1 showed little site fidelity with half of the 40 tagged animals leaving the farm within 5 days. A subsequent study of 62 lobsters in MB1 and MB2 showed that lobsters in the farms concentrated their activities in smaller areas than lobsters outside of the farm and moved at slower rates (Figure 2); the time lobster spent in farms was greater in MB1 than adjacent areas whereas the opposite trend was evident in MB2.

Lobsters did not remain within mussel farms or the area surrounding them, suggesting that they are available to the fishery and also that the farms do not act as ecological traps for the animals by concentrating them in the area and thereby making them more susceptible to the fishery.



FIGURE 1. Lobster fitted with acoustic tag

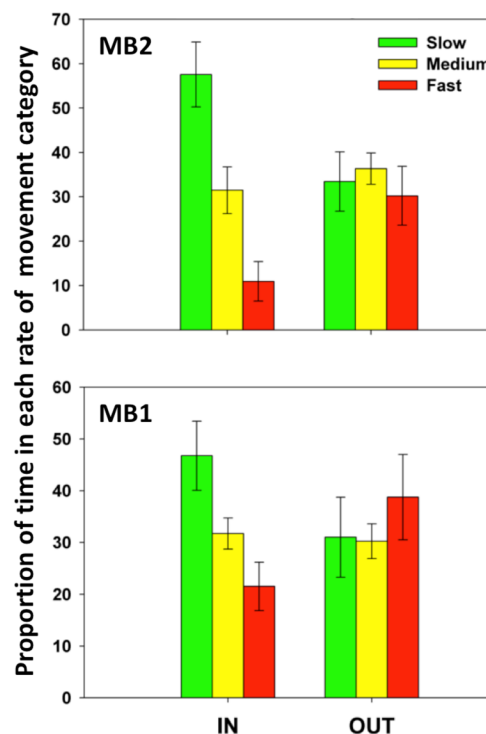


FIGURE 2. Rate of movement of American lobster inside and outside of mussel farms in Malpeque Bay.

UNTARGETED METABOLOMICS ANALYSES OF CULTURED DUSKY KOB *Argyrosomus japonicus* IN SOUTH AFRICAN AQUACULTURE

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The dusky kob (*Argyrosomus japonicus*) is known to suffer infections from the gill monogenean parasite *Diplectanum oliveri*. These monogenean parasites have adverse effects including epithelial hyperplasia and haemorrhage around attachment points and erosion and inflammation of gill lamellae. These often lead to secondary infections, reduced growth rates and mass mortality of fish stocks. To our knowledge, the physiological impacts of *D. oliveri* on farmed dusky kob have not been examined. The present study aims to identify and quantify metabolites in dusky kob which in turn can be used in applications of fish health assessment.

To achieve this objective, we applied GC-MS and LC-MS/MS based metabolomics analyses on fish dried blood spots (DBS) collected from four farms. The farms represent different parasite prevalence, geographical locations (KwaZulu-Natal, Eastern Cape and Western Cape) and farming conditions (RAS and ponds). A principal components analysis (PCA) was used as a reduction technique to cluster the samples based on similarities in metabolite concentrations. Using the identified metabolites, we produced a metabolomic profile for farmed dusky kob. Furthermore, we examined biological indices (hepato-somatic index and spleen somatic index), haematology parameters (blood glucose and packed cell volume) and condition factor from the fish.

A total of 25 organic acids, 20 amino acids and 8 acylcarnitines were identified with 6 amino acids (Citrulline, Glutamine, Lysine, Methionine, Phenylalanine and Proline) showed significant differences between the farms. The PCA produced a 43.5% variability (PC 1 and PC 2) which can be attributed to correlations in metabolite concentrations between the fish.

While significant differences in metabolite concentrations were observed and variations in metabolite correlations clustered the farms together (PCA), these observations cannot be attributed with certainty to parasitic infections only. Farming conditions, geographical locations and environmental factors have to be taken into consideration. Metabolomics is a valuable platform for fish health assessment as it may aid in early disease detection, biomarker development and implementation of corrective measures in farms.

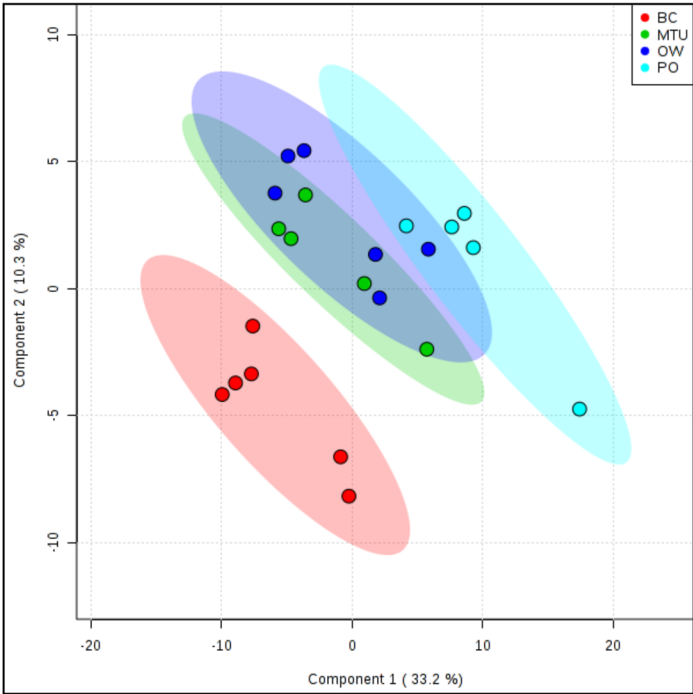


Figure 1: Principal components analyses scores plot for fish sampled from Blue Cap (BC), Mtunzini (MTU), Oceanwise (OW) and Pure Ocean (PO) fish farms.

Table 1: Various biological parameters measured from sampled fish. (CF = Condition factor; PCV = Packed cell volume; HSI = Hepato-somatic index).

| Farm       | Mass (g) | Glucose (mmol/L) | CF   | PCV (%) | HSI |
|------------|----------|------------------|------|---------|-----|
| Mtunzini   | 432.1    | 6.2              | 1.16 | 33.1    | 2.8 |
| Oceanwise  | 395.2    | 3.7              | 0.93 | 32.9    | 1.2 |
| Pure Ocean | 273.7    | 5.8              | 1.29 | 29.5    | 1.7 |
| Blue Cap   | 882.8    | 6.8              | 1.25 | 31.9    | 1.9 |

## **KENYA MARKET-LED AQUACULTURE PROGRAMME, CONSUMERS INSIGHTS MAKING MARKETS WORK FOR SMALL & MEDIUM SIZED POND FARMERS**

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Aquaculture is a nascent industry in Kenya; it was boosted by the ESP programme of Kenya that pumped 87 Million USD in promoting small scale aquaculture from 2009 to 2012. Kenya has 47 Million inhabitants and consumes 4.5 Kg of fish per person per year, while the African average is 9.7 kg per person per year. Fisheries cannot grow more in fresh, therefore the gap can only be covered by imports or aquaculture.

Only for sustaining the current level of consumption Kenya, taking into consideration only population growth, Kenya has to produce an additional 45,000 MT of fish in 10 years' time; sustainable aquaculture holds the key to filling this gap. But the big question is: how can a fish farmer benefit maximal from filling the gap? Current wholesale prices for tilapia are 3.5 USD/kg, which is above the average price in large producing countries, but farmers have a challenge tapping into the market. To solve the problem of pricing and market orientation KMAP, Kenya Market-led Aquaculture Programme (KMAP), funded by the Embassy of the Kingdom of The Netherlands (EKN) analysed the aquaculture value chain which included a consumer insight study and a value chain analysis.

To boost the aquaculture industry KMAP is working with the commercial oriented fish farmers and input suppliers, which are, in the Kenyan context, fish farmers with more than 900m<sup>2</sup> of fish ponds. Based on the market study the most profitable and easy to manage option is to supply the right size of fish in constant quantities to their nearby market.

The consumer study looked at regional consumption patterns, and perceptions about Catfish and Tilapia and was done by "informants" that asked in their network questions about aquaculture without the respondent knowing he is being interviewed. 44% respondents stating nutritional benefits, 35% taste. A comparison of wild vs farmed fish was also made, but it was established that the vast majority of consumers cannot tell whether its farmed or wild.

The project tries to relate the consumer preferences and the marketing approaches that can be used by traders already linked to KMAP farmers specifically in the western region, to promote consumption of catfish which is easier and more economical for farmers to rear. With the insights gathered so far, the project will promote local marketing channels since there the sales prices are higher and logistical costs lower.

## PERIPHYTON CHARACTERISTICS INFLUENCE THE GROWTH OF *Holothuria scabra* JUVENILES IN AN OCEAN NURSERY SYSTEM

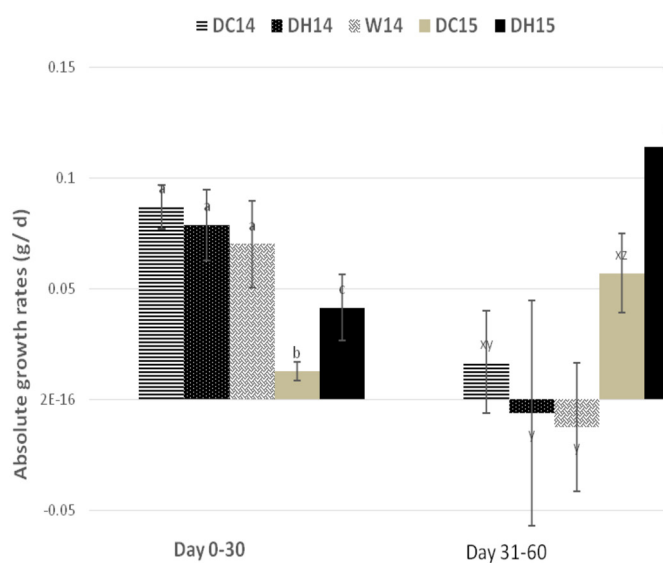
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Floating *hapas* (fine mesh enclosures) are a cost-effective ocean nursery system to culture post metamorphic *Holothuria scabra* to release size (>3 g). The growth of periphyton on *hapas* is a natural food source for early juveniles. This study investigated the temporal effects of periphyton variation on the growth and survival of juvenile *H. scabra* reared in floating *hapas*. Five trials where juveniles were reared for 60 days each in the eutrophic coastal waters of Bolinao, Pangasinan were conducted during different seasons: dry cold (DC), dry warm (DW) and wet (w) in 2014-15. The periphyton biomass parameters monitored were chlorophyll-a (diatom biomass), ash-free dry weight (AFDW), their derivative, autotrophic index (AI) and phaeopigment.

Average weight (g) and AGR (g/d) for the first 30 days, and were significantly higher than the 2015 runs ( $p < 0.05$ ). Juveniles reared during DC15 had the lowest average weight ( $0.38 \pm 0.13$  g), and lowest AGR ( $0.01 \pm 0.004$  g/d). At the end of 60 days, juveniles reared during DH15 had the significantly highest average weight ( $4.67 \pm 1.05$  g), and highest AGR ( $0.08 \pm 0.017$  g/d). Juveniles in DH14 and W14 had negative AGR from day 30-60, which were statistically similar with DC14.

Good growth ( $\sim 0.07$ - $0.09$  g day<sup>-1</sup>) during the first 30 days of rearing was characterized by higher diatom abundance, and lower AI, while lower chlorophyll-a and higher AI was related to poorer growth ( $\sim 0.01$ - $0.04$  g day<sup>-1</sup>). Conversely, the last 30 days of rearing, good growth ( $\sim 0.06$ - $0.11$  g day<sup>-1</sup>) was associated with higher AI and lower diatom abundance, while a higher diatom abundance and lower AI was related to poorer growth ( $\sim 0.01$  g day<sup>-1</sup>) during both years. This study underlines the importance of water quality during the rearing of juvenile *H. scabra* in floating *hapas*, as well as their potential shift in feeding from grazers to detritus feeders as they grow.



## EFFECTS OF SEASONAL ENVIRONMENTAL CHANGES ON CAGE FISH PRODUCTION IN LAKE VOLTA, GHANA

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Globally, aquaculture production is faced with numerous environmental challenges, notable among which is water quality which has to be suitable throughout the production cycle to ensure a successful operation. The climate of Ghana is characterized by two seasons: the dry and wet seasons which are known to influence the quality of aquatic environments. The study determined possible seasonal changes in water quality of the Lake and how the changes, if any, could affect the production of *Oreochromis niloticus* in cages in the Lake.

Three cages, each measuring 81 m<sup>3</sup>, were set in the Lake and stocked with 22 g fingerlings at a rate of 40 m<sup>-3</sup> per cage and fed commercial diet. The growth performance and selected water quality parameters were monitored in both dry and wet seasons between November, 2014 and June, 2015. The yield from each season was determined. Besides the experimental set up, similar information was obtained from a commercial farm nearby within the same period and for both seasons.

There were some differences in water quality parameters between the dry and wet seasons but were not significant (Table 1) ( $p > 0.05$ ). Water quality for both seasons remained within the limits required for good tilapia growth in both commercial and experimental farms.

Growth characteristics determined also showed minimal variations between seasons with the final mean weight for the dry season being slightly higher (Table 2). The difference in the final mean weight was because fish cultured in the dry season were better feed converters resulting in a slightly higher yield (Table 2). Similar result of higher yield in the dry season was also obtained in the commercial farm.

Table 1: Mean water quality results for the dry and wet seasons in the experimental farm

| SEASON     | Temp (°C)    | DO (mg l)   | pH(pH units)             | TURB (NTU) | NO <sub>2</sub> -N (mg l) | NO <sub>3</sub> -N (mg l) | PO <sub>4</sub> -P (mg l) | NH <sub>4</sub> (mg l) |
|------------|--------------|-------------|--------------------------|------------|---------------------------|---------------------------|---------------------------|------------------------|
| DRY SEASON | 29.86 ± 0.29 | 5.29 ± 2.07 | 6.94 ± 0.12 <sup>a</sup> | 4.40±1.85  | 0.008±0.007               | 0.052±0.044               | 0.139± 0.050              | 0.227±0.203            |
| WET SEASON | 29.02 ± 0.75 | 6.10 ± 0.70 | 6.98 ±0.29 <sup>a</sup>  | 2.80±1.33  | 0.029±0.016               | 0.051±0.016               | 0.062±0.040               | 0.094±0.076            |

Table 2: Growth parameters monitored in both dry and wet seasons of fish culture in experimental farm

| GROWTH PARAMETER                            | DRY SEASON                  | WET SEASON                  |
|---|-----------------------------|-----------------------------|
| Initial mean wt. (g)                        | *22.49 ± 0.04 <sup>a</sup>  | 22.70 ± 0.05 <sup>a</sup>   |
| Final mean wt. (g)                          | 311.39 ± 24.84 <sup>b</sup> | 283.77 ± 4.79 <sup>b</sup>  |
| Specific growth rate (g day <sup>-1</sup> ) | 1.88 ± 0.06 <sup>a</sup>    | 1.89 ± 0.01 <sup>a</sup>    |
| Mean daily wt. gain (g day <sup>-1</sup> )  | 2.06 ± 0.18 <sup>a</sup>    | 1.92 ± 0.04 <sup>a</sup>    |
| Condition factor (g cm <sup>-1</sup> )      | 3.99 ± 0.17 <sup>a</sup>    | 3.95 ± 0.04 <sup>a</sup>    |
| Net Yield (kg)                              | 715.10 ± 99.35 <sup>a</sup> | 707.93 ± 90.85 <sup>a</sup> |
| Food conversion rate                        | 1.34 ± 0.16 <sup>a</sup>    | 1.57 ± 0.10 <sup>a</sup>    |
| Survival (%)                                | 86.48 ± 12.90 <sup>a</sup>  | 76.73 ± 6.35 <sup>a</sup>   |



# **MORPHOLOGY OF THE ALIMENTARY CANAL AND FEEDING INTENSITY OF *Horadandia atukorali* DERANIYAGALA1943 (CYPRINIDAE), AN INDIGENOUS ORNAMENTAL BARB OF THE WESTERN GHATS OF INDIA**

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*Horadandia atukorali*, Deraniyagala, 1943 is a very small indigenous ornamental cyprinid fish commonly known as 'Glow light carplet', or 'Green carplet' that is found in the Western Ghats of India and Sri Lanka. It is a much sought after fish in the international ornamental fish market. It is the only member of its genus. It inhabits still or slow-moving water, frequenting swamps, rice fields and similar still waters. It has a terminal and oblique mouth with small gape, suggested that it is a column feeder. Teeth were absent in the jaws and buccal cavity. The alimentary canal which originated from mouth to the anus was a small tapering tube. They belonged to the group of stomach-less fishes in which the digestive tube consisted of mouth, pharynx, oesophagus, intestine and anus. Pharynx was divided into two parts anterior respiratory with gill rakers and gill filaments and posterior masticatory part. The posterior region consisted of pharyngeal teeth in two rows with pharyngeal formula 4, 2/2, 4, with 4 or 5 terminal cusps. Histological studies showed that the structure of the alimentary canal was uniform throughout the length except for the variations in the number and depth of mucosal folds, shape of the cells and thickness of muscular layer. It is a column feeder, mainly a carni-omnivore as well a micropredator feeding on small insects, worms, crustaceans and other zooplanktons along with small amount of phytoplankton. Feeding intensity was explained by describing the guts in different degrees of fullness. The data of percentage occurrence of guts in different degrees of fullness in males and females from two locations were studied. Monthly variation in gastro-somatic index of male and female from two locations were studied. The relative gut length were calculated in males, females and juveniles and it ranged between 0.6 and 0.95 in males, 0.5 and 0.7 in females and 0.5 and 0.7 in juveniles respectively.

## **BIODIVERSITY OF INDIGENOUS ORNAMENTAL FISHES OF THE WESTERN GHATS OF INDIA-STATUS AND PROSPECTS**

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The Western Ghats (WG) bio geographic region in southern India runs along the west coast extending from 08°19'08"–21°16'24"N to 72°56'24"–78°19'40"E with a north to south distance of 1,490 km, a minimum width of 48 km and maximum width of 210 km, covering a total area of 136,800 km<sup>2</sup> (CEPF 2007). The WG mountain range traverses the states of Gujarat, Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu interrupted only once by a 30-km break called the Palghat Gap in northern Kerala. The freshwater rivers and streams in the WG fall under five main ecoregions, viz., Narmada-Tapi, the Northern Deccan Plateau (Godavari River system), the Southern Deccan Plateau (Krishna River system), the Southern Eastern Ghats (Cauvery River system) and the Western Ghats (west flowing rivers). The freshwater ecosystem and all its denizens together constitute the rich biodiversity of one the world's 34 hotspots. The freshwater ecosystem biodiversity within the WG region is highly diverse, unique and of immense importance to livelihoods and economies. It is a home to some of the world's most unique fauna, flora and fungi. Compared to the other hotspots, it has the highest human population per unit area (more than 300 humans/km<sup>2</sup>), making it that much more challenging to conserve (Molur 2009). The Western Ghats have also lost nearly 50% of forest cover since the early 1900s and the trend is continuing with increased fragmentation and encroachments. The WG is very rich in faunal diversity with 300 species of freshwater fishes. The endemism is the highest in amphibians (78%), followed by reptiles (62%) and fish (53%). In the present paper the status and distribution of the fresh water fishes with special reference to the indigenous ornamental fishes is discussed. Of the 300 species of fishes 155 have ornamental value and 50% of them are endemic.

## BEHAVIORAL PYREXIA AS AN IMMUNE RESPONSE IN *Argyrosomus japonicus* (PISCES: SCIAENIDAE)

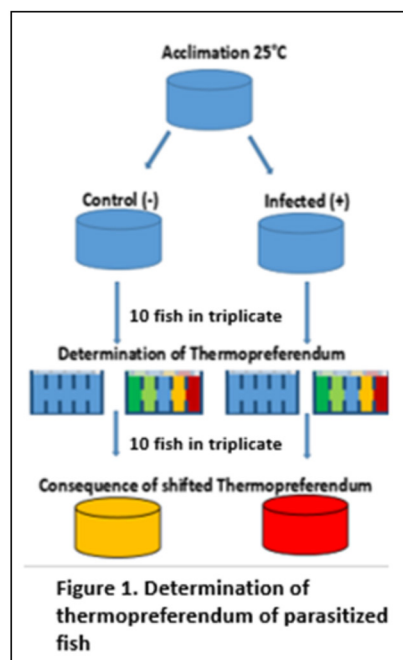
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Parasites are a major cause for concern in aquaculture systems as they lead to fish disease which has a negative impact on the income that can be generated by any aquaculture facility. Disease can impact income directly by causing mortalities or indirectly by causing a change in behavior, negatively affecting the growth rate or making fish susceptible to other environmental stressors. Treatment and monitoring of parasites is therefore imperative for any aquaculture facility.

Behavioral fever is the phenomena where ectotherms select a higher environmental temperature in order to raise core temperature and induce a fever. It has been reported in several fish species in response to pathogens and has been shown to improve the immune response in fish. The use of behavioral fever in aquaculture to enhance the immune response against pathogens will eliminate the need to use harmful chemicals such as hydrogen peroxide, formaldehyde and potassium permanganate to treat pathogens. This study aims to investigate behavioral fever in *Argyrosomus japonicus* that has been challenged with a scuticociliate parasite and determine if the fever response will boost the immune response.

To achieve this fish were challenged with a scuticociliate parasite and then placed in a thermal gradient tank (20-28°C) that allowed fish to select a preferred temperature. Thermal preference was determined by visual observations, videography and the Lolitrak V4 software which tracked behavioral patterns of individual fish. Fish were then kept at their preferred temperature for 7 days to determine the effect of the changed thermopreferendum. The response to the shifted thermo preferendum was measured non-destructively (hematocrit, blood glucose, metabolic rate, condition index) and after euthanasia (histology, cortisol, prostaglandin and iL-1 $\beta$  mRNA transcripts).



## GENOTYPIC AND MORPHOLOGICAL VARIATION IN THE COMMERCIAL CULTURE OF BLUE MUSSELS *Mytilus edulis*

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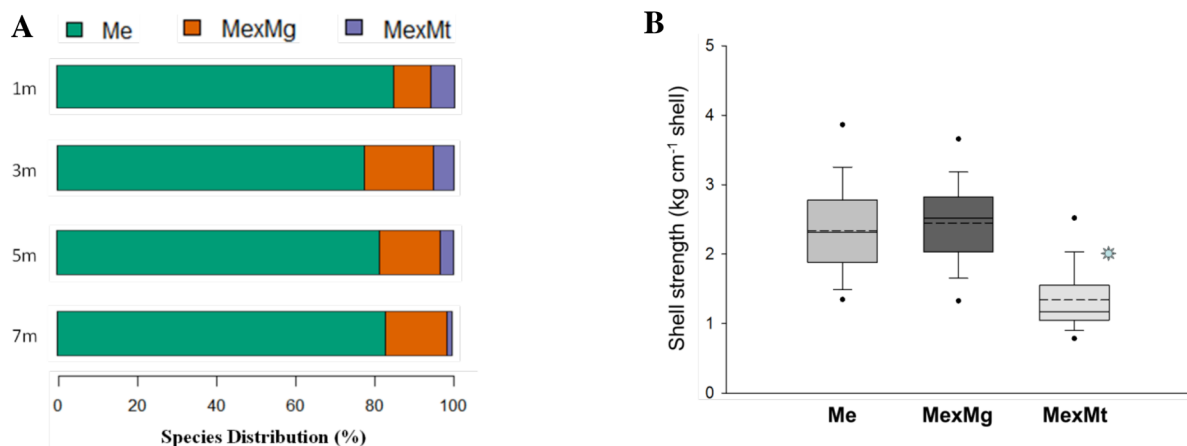
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In the Northern Hemisphere, the commercially important blue mussel complex is composed of three closely related species: *Mytilus edulis* (Linnaeus, 1758), *M. galloprovincialis* (Lamarck, 1819) and *M. trossulus* (Gould, 1850). To date, their definitive taxonomic discrimination using morphological characteristics alone has been difficult due to high phenotypic plasticity, further complicated by hybridisation in areas of sympatric occurrence. By relating results of genetic species identification to shell morphological features, we show a morphological identification method for species within the blue mussel complex. We demonstrate an accurate and cost-effective strategy to identify *Mytilus* species at cultivation sites, including the commercially damaging fragile shelled *M. trossulus*.

Adult mussels (2 - 2.5 yrs) were sampled over one year (Jun 2015 – Jun 2016), originating from different depth at a longline mussel farm on the Scottish west coast. Individuals ( $n = 440$ ) were genotyped using a single nuclear marker Me15/16, and their shell strength and shell shape analysed. We found variation in *Mytilus* genotype distribution down the length of a single cultivation rope with hybrids of *M. trossulus* being more abundant in the surface (5.9 % at 1m) and less frequent at greater depth (1.2 % at 7m; Fig 1A). In addition, shells of *M. trossulus* hybrids were weaker ( $1.34 \pm 0.43 \text{ kg cm}^{-1}$  shell length) than those of non-*trossulus* mussels, with  $2.37 \pm 0.75$  and  $2.45 \pm 0.62 \text{ kg cm}^{-1}$  shell length for pure *M. edulis* and their hybrids with *M. galloprovincialis*, respectively (Fig 1B). Both shell strength and shape varied with the mussels' position on the rope, with generally stronger and rounder shells with increasing depth. The spatial variation in *Mytilus* species distribution is partially governed by environmental conditions encountered, with salinity in particular previously suggested to favour *M. trossulus*.

This affordable and quick identification method may lower the risk of translocations of less desired *Mytilus* species and improve management strategies for the local mussel industry.



**Fig 1A:** *Mytilus* species distribution down a mussel cultivation rope. **B:** *Mytilus* genotype in relation to shell strength. Me: *M. edulis*; MexMg: hybrids of *M. edulis* and *M. galloprovincialis*; MexMt: hybrids of *M. edulis* and *M. trossulus*. Asterisk indicates significantly lower shell strength in MexMt hybrids.

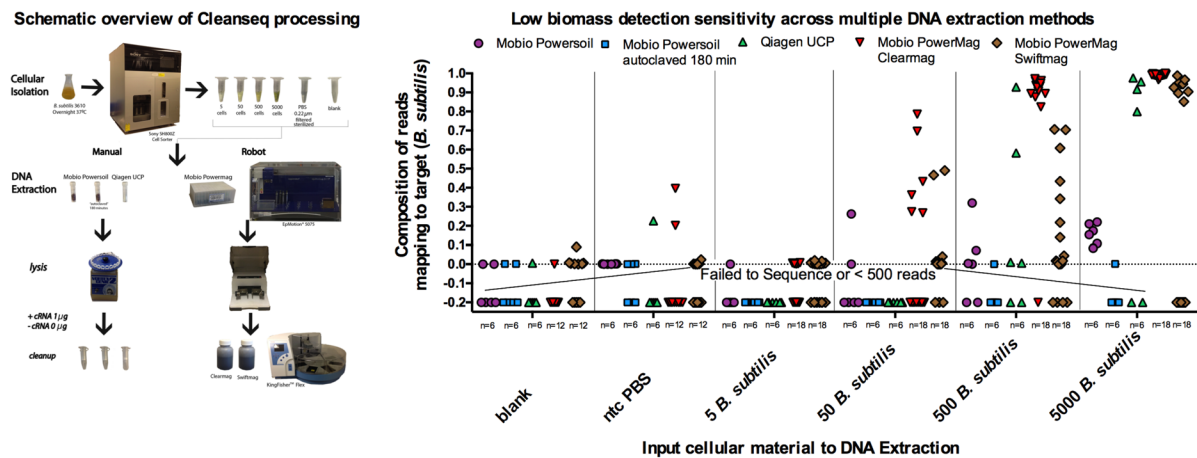
CLEANSEQ ENABLES HIGH-THROUGHPUT MICROBIOME ANALYSIS FROM LOW BIOMASS SAMPLES AND IS APPLIED TO IDENTIFY SPATIAL DISTRIBUTION OF MICROBES FROM THREE CLEANROOMS

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Rapidly identification and monitoring of disease outbreaks in clinical cleanroom environments such as hospitals or aquaculture facilities is essential for preserving life and preventing catastrophic economic loss due to massive deaths. Microbiome processing and analyses of low-biomass samples are challenging because of contamination and inefficiencies, leading many investigators to employ low-throughput methods with minimal controls. We introduce a high-throughput low-biomass pipeline (CleanSeq) that reveals the whole bacterial community from inputs as little as 500 cells using automated robotic platforms which we determined by comparing samples processed by 5 different methods (n=300). We apply this method to both the 16s and metagenomics analysis of the JPL spacecraft assembly facility (n=192, 96), Neonatal Intensive Care Unit (n=480, 384), and a critically endangered abalone rearing facility with monitoring of withering syndrome (n=192, 192) revealing spatially resolved, distinct microbiomes, reproducible across hundreds of samples from various built environments.

Figure 1: Low biomass microbiome kit evaluation. (a) Experimental design of low biomass kit evaluating negative and positive controls (5, 50, 500, and 5000 bacterial cells) across three solid phase and two magnetic bead based DNA extraction methods. (b) Amplicon libraries of 16S amplicons were sequenced and compared against reference to determine effective limit of detection across kits. The limit of detection was lowest for the Mobio Powermag clearmag kit, identifying 500 cells at 92% accuracy.



# TILAPIA, *Oreochromis shiranus*, AND AFRICA CATFISH, *Clarias gariepinus*, REARED TOGETHER IN PONDS FERTILIZED WITH VARIOUS TYPES OF LIVESTOCK MANURE HAVE STABLE AND UNIQUE MICROBIAL COMMUNITIES, UNAFFECTED BY MANURE TYPE

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The majority of global seafood production has shifted from wild caught to farmed, with inland, freshwater systems generating over 87 % of all cultured finfish. Growth in annual aquaculture production is greatest in Africa at 11.7 %, yet improving production and efficiency for rural farmers while ensuring safety for human consumption is needed. Most fish farms in Malawi are small to medium scale earthen ponds fertilized using livestock manure with *Oreochromis shirnaus* and *Clarias gariepinus* reared in polyculture. To understand the probiotic or prebiotic effects of livestock manure in polyculture systems, we grew tilapia and catfish together for four weeks under 7 manure treatments including layer chicken, broiler chicken, guinea fowl, quail, pig, cow, and standard commercial feed only. We evaluated the microbial communities of the manure, water column, tilapia gut, and catfish gut using 16s and 18s marker genes along with whole genome shotgun sequencing. Microbial diversity was greatest the water column and tilapia gut followed by catfish gut and input manure having the lowest. Manure types had highly variable eukaryotic and prokaryotic microbial communities but contributed less than 10 % of the microbes to the fish guts. Catfish and tilapia reared in the same tanks had vastly differentiated microbial gut communities with tilapia sharing many more microbes with the water column such as cyanobacteria, which is likely due to differed feeding ecologies. Catfish growth however, was most strongly positively correlated with microbial activity suggesting future development in probiotic treatment is needed particularly at the larval and fry stage. In this experimental mesocosm study we demonstrate how the composition of fish pond microbiomes are not altered by livestock manure inputs and verify this lack of microbial transmission to farmed fish. We identify species specific manure preferences for blue tilapia and African catfish to be broiler manure and quail, respectively.

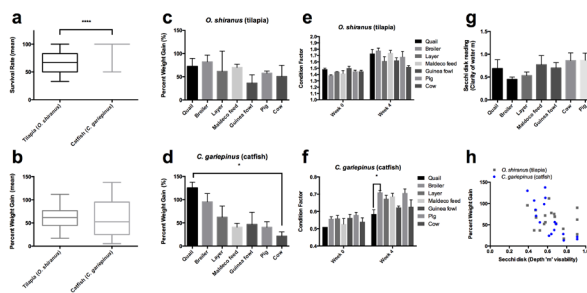


Figure 1. Manure treatments effects on *C. gariepinus* and *O. shiranus* production

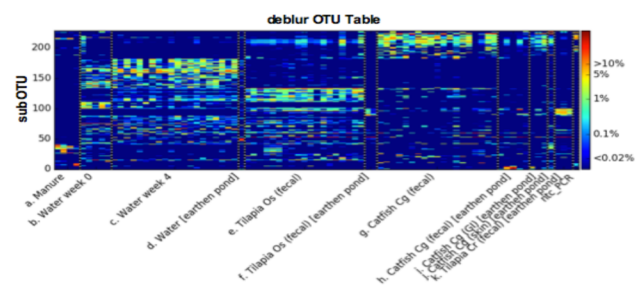


Figure 2. 16s Analysis of fish pond microbial communities

**STATUS OF MARICULTURE IN KENYA “IMPROVED LIVELIHOOD AND FOOD SECURITY”**

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Mariculture development in East Africa (EA) is dependent on wild seed supply and under developed. The history of mariculture in the region can be traced back to the earlier 1980's when commercial interventions were initiated in Kenya. Community based small scale mariculture was introduced about two decades ago along the coast of Kenya and organized community groups (OCGs) formed the entry point. Many of the OCGs were either formed by NGOs to specifically implement funded projects or were developed by communities out of influence from neighbour colleagues benefiting from similar organizations or just change of community merry go-round self-help groups. Significant changes have been observed in a number of OCGs that have increased by 360% between 2007 and 2016 while the area under pond culture increased by 504 %, crab cages by 204 % while fish cages and seaweed farming (currently 144 farmers) has also been introduced during these period. Target species for culture involve; milkfish, mullets, mud crab, prawns and currently seaweed and artemia. Production capacity has varied between 0.06-0.2kg fish/m<sup>2</sup>, 0.02-0.03kg prawn/m<sup>2</sup> and 5-20kg crab/month in 2007 and 0.08-0.45kg fish/m<sup>2</sup>, 0.02-0.05kg prawns/m<sup>2</sup> and 15-40kg crab/month and 0.15 – 0.35 tons of seaweed/farmer in 2016. Irrespective of the significant growth of the industry and its contribution as a livelihood option; the farming has been characterized with low and inconsistent annual production regimes that has been associated to among other things; limited seed supply, technology adoption, ownership of interventions, rules of engagement, business aspect of interventions, group management and conflict resolutions mechanisms.



## **EVALUATING THE PERFORMANCE OF FRESHWATER NILE TILAPIA (*Oreochromis niloticus*) FROM EAST AFRICA AT DIFFERENT SALINITIES AND ON DIFFERENT FORMULATED FEEDS**

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Aquaculture is one of the fastest growing animal food production sectors in the world and consequently, sustainable aquaculture is emerging as an increasingly critical component of the Blue Economy globally. Freshwater aquaculture has significantly grown in the East Africa region through development of high value species like Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*), whose seed is readily produced locally in hatcheries while markets are at both a local and an international scale. Conversely, mariculture has not yet developed to realize its economic potential, with the exception of seaweed farming. Other species like milkfish and prawns are farmed at a subsistence level. The marine environment has many fish species of high economic value but hatchery facilities to produce seed are expensive and thus missing which leads to unreliable dependency on wild seed. The high infrastructure and operating costs being a major impediment to investment in hatcheries.

In an effort to jumpstart the mariculture sector into commercial viability in East Africa, innovative research on the viability of farming Nile tilapia at different salinities and when fed using formulated feeds including a novel feed ingredient, Novacq<sup>TM</sup>, was evaluated for the first time in East Africa. Nile tilapia seed was produced locally with borehole water (0.63ppt salinity) and grown to 1.0 – 2.4 cm total length. The tilapia seed were taken through several increasing salinity experiments in the laboratory at KMFRI using glass aquaria between 5ppt – 30ppt over a period of 7 days at each salinity level. After acclimatizing at 30ppt salinity they were transferred to brackish water earthen ponds with salinity variations of 28ppt – 33ppt where they were stocked in net cages within the ponds. Within the net cages, the fish were fed on three different formulated diets (Novacq<sup>TM</sup> feed, No Novacq<sup>TM</sup> control diet, and a reference local community-formulated feed) for a period of 3 months where growth and survival were estimated.

This paper will present results for survival and growth of Nile tilapia when reared in the different salinity treatments in the laboratory, and when fed on the three different diets within the net cages. This research will be critical for assessing the potential of Nile tilapia as a relatively high value species for farming in brackish water for local coastal communities with limited resources to develop elaborate marine hatchery systems. Furthermore, this research will provide initial data to evaluate the cost-effectiveness of feed formulations using Novacq<sup>TM</sup> for community-based farming in East Africa.

## ASSESSMENT ON ABUNDANCE AND CHEMICAL COMPOSITION OF LOCAL FEEDSTUFFS USED BY TILAPIA FISH FARMERS IN TANZANIA

Francis Pius Mmanda<sup>1</sup>, Deogratius Pius Mulokozi<sup>2</sup>, Jan Erik Lindberg<sup>\*\*</sup>, Torbjörn Lundh\*, Anna Norman Halldén\*, Matern Mtolera\* and Rukia Kitula\*

Currently, there are an increasing number of commercial aqua-farmers in Tanzania, from 21300 earthen fish ponds in 2014 to 22,785 earthen fish ponds in 2015 (URT, 2015). The continuous increases lead to high demand of good quality aqua-feeds and fish seeds. Despite of the huge potential we have and post government support, aquaculture production in Tanzania is negligible. The production increases from 3118MT (2014) to 3239.99 MT in 2015 (URT, 2015). Although there are many unknown fish feeds producers, only one known fish pellets producers in the country. Despite of their high price (1.5USD per Kg) but the impacts of their feeds produced and supplied to tilapia growers are not yet realized. Meanwhile poor growth rate retardation condition have been experienced by aqua-farmers due to improper feed fed, poor quality of feed supplied or offering nutrient deficit feed to fish. In response to this situation, several studies have been done to investigate the effects of feed formulated on fish growth performance and nutrients utilization. However, the information about abundance and nutrients composition in Tanzania are lacking. Thus the field survey on assessment of abundance and chemical composition of local feedstuffs used by tilapia farmers in Tanzania is currently undertaking in the aquaculture potential areas in order to evaluate various types of traditional local feedstuffs used to feed fish in aqua-farms in various parts of the country as well as to evaluate their nutrients composition.

The results of this study will provide guidance for fish feed producers (factories/ companies) and hatcheries operators, as well as aqua-farmers of both small and large scale operation systems. Hence, the study will provide data for possible solutions of one of the major challenges (i.e. good quality feeds) facing aquaculture industry in Tanzania today and for several decades.

We are expecting to complete our study before May, 2017. Thus I would like to present my results to the conference as Poster presentation. However I did some research on study of streptococcal infection associated with *Streptococcus iniae* in a cage farmed fish, *Sciaenops ocellatus* in eastern China Sea but data were not presented here.

## GROWTH RATES OF SELECTED *Oreochromis* SPECIES CULTURED AT DIFFERENT SALINITIES

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Tanzania has a variety of *Oreochromis* species some of which have proven tolerance to high salinity. Unfortunately, the best species with proven track of success in aquaculture including high growth rate, *O. niloticus*, has a poor tolerance to salinity. Experiments were carried out to find an ideal *Oreochromis* species for aquaculture in estuarine and marine environment. Three studies were carried out on growth rates of *Oreochromis* sp using *O. urolepis urolepis*, *O. pangani*, and crossbreeds of *O. urolepis urolepis* supper ♂ and *O. niloticus* ♀.

The experiments were carried out at the designated Institute of Marine Sciences Mariculture Centre at the Pangani Estuary, Tanga, Tanzania. Except for *O. Pangani* where fingerlings were collected from Pangani River, the others were hatched at the Mariculture Centre and raised from fry. The fish were acclimatized at a rate of 2 salinity units per day to the salinities of 15, 25 and 35 using freshwater as a control. The feed for fry was formulated at 40% protein and fed at 10% of their body weight divided to 3 portions fed at 8:00, 12:00 and 16 hrs, while the feed for the fingerlings was formulated at 20 – 28% crude protein (pelleted) and fed at 5% body weight divided into 2 portions fed at 8:00 and 16:00 hrs. After acclimatization the fish were raised for 8 weeks. The parameters studied were final weight, Average Daily Gain (ADG), Specific Growth Rate (SGR), and survival rate (SR). Environmental parameters and proximate analysis were also done for control purposes.

The results (Table 1) indicated that the hybrids had the highest growth rates followed by *O. urolepis hornorum* and *O. Pangani*. On the individual species the highest growth rate was exhibited at salinities of 25 for the three species. While the specific growth rate were significantly different at a salinity of 25 for *O. urolepis urolepis*, there was no significant differences across salinities for *O. Pangani* and the hybrids. The survival rates were high at all salinities despite the tendency of becoming slightly lower at 35. The higher growth rate in brackish water salinities may be related to isotonicity between the media and the body fluids. The hybrids produced were found to be all males. These characteristics including production of all males can be exploited to farm tilapia in the marine environment.

Table 1. Fry and Fingerlings Growth and Survival Parameters

| Species                     | Salinity | ADG (g) | SGR (%) | SR (%) |
|-----------------------------|----------|---------|---------|--------|
| <i>O. Pangani</i>           | 0        | 0.17    | 1.83    | 100    |
|                             | 15       | 0.28    | 1.21    | 100    |
|                             | 25       | 0.27    | 1.83    |        |
|                             | 35       | 0.22    | 1.73    | 90     |
| <i>O. urolepis urolepis</i> | 0        | 0.007   | 2.47    | 96     |
|                             | 15       | 0.008   | 3.07    | 97     |
|                             | 25       | 0.031   | 4.86    | 93     |
|                             | 35       | 0.35    | 3       | 98     |
| Hybrid                      | 0        | 0.24    | 6.35    | 87     |
|                             | 15       | 0.35    | 6.41    | 86     |
|                             | 25       | 0.39    | 7.02    | 91     |
|                             | 35       | 0.36    | 6.12    | 87     |

## NITROGEN AND PHOSPHORUS REMOVAL FROM BREWERY EFFLUENT USING MICROALGAE

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Microalgae have been mass cultured to fill a variety of roles, such as in wastewater treatment. Microalgae were cultured in ponds, known as high rate algal ponds (HRAPs) for tertiary treatment of anaerobically digested brewery effluent at Ibhayi Brewery at an experimental scale. The microbial community structure and physicochemical parameters in the HRAPs were investigated over a period of 12 months. Furthermore, for a better understanding of the HRAPs dynamics, an experiment was conducted in a controlled environment. Microalgae were cultivated under different temperatures (20 and 30) and different pH levels (7.0, 8.5 and 10.0). Two pH adjustment methods, carbon dioxide (CO<sub>2</sub>) and hydrochloric acid (HCl) were used. The Chlorophyll *a* (Chl *a*), ammonium-nitrogen (NH<sub>4</sub><sup>+</sup>-N), nitrite-nitrogen (NO<sub>2</sub><sup>-</sup>-N), nitrate-nitrogen (NO<sub>3</sub><sup>-</sup>-N) and Orthophosphate-phosphorus (PO<sub>4</sub><sup>3-</sup>-P) concentrations were measured and served as indicators of the HRAP's performance.

The Chl *a* concentration increased with an increase in temperature, with the maximum Chl *a* concentration at both temperatures obtained at pH 8.5. The removal of NH<sub>4</sub><sup>+</sup>-N and PO<sub>4</sub><sup>3-</sup>-P also increased with an increase in temperature. The removal of NH<sub>4</sub><sup>+</sup>-N was greater at pH 10.0 than at pH 7.0 and 8.5 when cultures were incubated at 20 and 30. There was a significant difference ( $p < 0.001$ ) in NH<sub>4</sub><sup>+</sup>-N removal at all pH levels when using both pH adjustment methods. At 20, the main mechanism of NH<sub>4</sub><sup>+</sup>-N removal was through microbial activity (algal assimilation and nitrification) at all pH levels. However, at 30, a greater portion of NH<sub>4</sub><sup>+</sup>-N was removed through microbial activity at pH 7.0 and 8.5, whereas at pH 10.0, volatilization was the main mechanism of NH<sub>4</sub><sup>+</sup>-N removal. The PO<sub>4</sub><sup>3-</sup>-P concentrations decreased significantly ( $p = 0.02$ ) at all pH levels in the CO<sub>2</sub> method, however, in the HCl method, there was no significant difference ( $p = 0.44$ ). Orthophosphate-phosphorus removal from the effluent appeared to be due to algal assimilation. This could be supported by the increase in PO<sub>4</sub><sup>3-</sup>-P removal which concurred with higher Chl *a* concentrations in the CO<sub>2</sub> method than in HCl method.

The outcomes of this experiment provided an understanding of the mechanisms responsible for nutrient removal in the HRAPs. The removal of NH<sub>4</sub><sup>+</sup>-N was through both microbial activity and volatilization of NH<sub>3</sub>-N, whereas, PO<sub>4</sub><sup>3-</sup>-P removal appeared to be through algal assimilation.

## PARASITIC CRUSTACEANS *Lernaea cyprinacea* and *Argulus japonicus* AND THE POSSIBLE ROLE THEY PLAY IN THE DEVELOPMENT OF AQUACULTURE IN THE FREE STATE PROVINCE

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Inland aquaculture has started to receive increased interest in the Free State, as a means of contributing to food security, creating jobs and diversifying agriculture production. Sharptooth catfish/baber (*Clarias gariepinus*), common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*) and Mozambique tilapia (*Oreochromis mossambicus*) have been identified as being economically viable species. Under aquaculture conditions, susceptible hosts are in close proximity, thereby facilitating the transmission and establishment of parasites in aquaculture systems. Therefore diseases and parasites of fish are regarded as significant constraints to the development of aquaculture in this region. The present study is a disease and parasite survey of commercially important fishes of the Free State, wherein we qualitatively assess the risk to the sustainable development of a freshwater fish aquaculture industry based on parasitic data collected from field surveys in the Free State Province carried out over two years and from scientific literature. The taxa collected included monogeneans and peritrich ciliophorans, the cestode *Schyzocotyle acheilognathi* and alien parasite species such as *Trichodinella epizootica*, *Argulus japonicus* and *Lernaea cyprinacea*. For the purposes of this presentation the focus will be on *A. japonicus* and *L. cyprinacea*, where 195 specimens of *L. cyprinacea* and 155 specimens of *A. japonicus* were found infesting four different fish host species. *Lernaea cyprinacea* and *Argulus japonicus* have been shown to cause clinical disease under aquaculture conditions as well as in natural systems.

## TOWARDS OPTIMIZATION OF WIND HYBRID SOLAR RENEWABLE ENERGY POTENTIAL ASSESSMENT AS AN ENGINE FOR SUSTAINABLE DEVELOPMENT IN KENYA

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Kenya straddles on a coastline of 640km with an EEZ of 390km rich in aquatic marine resources which remains under-exploited due to lack of cheap, clean unreliable electricity, yet it lies in the wind and sunshine belt. Globally, world resources of fossil fuel face threat of depletion and contribute in greenhouse emissions, any effort to harness the wind and solar energy, which is inexhaustible and environmental friendly, will accelerate development and make Kenya an economic commercial hub. An eighteen month 24 hr periodical cycle study of an interval of one hour on cut-in wind speed peak dynamics was conducted at English point Tudor creek between December 2014 to May 2016. The aim of the study was to determine the strength of the wind through the seasons at the site. An innovative method was used where a cloth and balloon were taken to mimic the conventional anemometer. The speed of the wind was derived from the angle subtended by the cloth. The cloths were tied on three mangrove posts, approximately 4meters tall, in a periodical sequence of two, three and four replicated thrice. The degrees subtended were derived using Bourfot Ranking Scale. Accuracy of the data was validated using the balloon method. The solar radiations were also recorded on an hourly basis, daily based on the solar insolation aspect. Other parameters taken include humidity, wind direction, tidal rhythms and the weather conditions. A total of 19,128metrics were taken for 676days. Mean site cut-in wind speed was  $6.113\text{ms}^{-1}$  in the Kaskazi North East monsoon Winds (November- March/April) and  $12.972\text{ms}^{-1}$  for the Kusi- South East Monsoon Winds (April/May to October). The data was processed and analyzed using SPSS and graphically presented as given below. From this study it is revealed that the coastal region is of great potential for generating renewable energy for exploiting the aquatic resources which are in great abundance but under-exploited both inshore and offshore.

### Introduction

Energy is fundamental to the quality of life on the earth. It is a key ingredient in all sectors of modern economics. Ordinarily, on a day to day basis we are totally dependent on energy be it for living or working. Therefore supply should be uninterrupted and abundant. Meeting the global demand growing demand sustainably is one of the challenges facing the industry. By 2050, the world's population is set to increase from 6.6billion to more than nine billion. Energy use during this semester in time is set to double. Due to human population geometric growth, industrial establishments, wanton destruction of forests Energy is a critical input for the achievement of many of the Sustainable Development Goals, including eradicating extreme poverty and hunger, achieving universal primary education, promoting gender equality and empowering women, reducing child mortality, improving maternal health, combating diseases, and insuring environmental sustainability. For adults and children, gaining access to reliable energy raises the living standard and income generation. Solution to energy needs can transform lives of billions, many of whom spend hours collecting and transporting firewood and other forms of biomass and all of whom are exposed to household air pollution from solid fuels which killed an estimated 3.5million and caused many more cases of respiratory, cardiovascular illnesses in 2010 (Lim SS 2012). This energy can also be used in modern cooking methods cold chain processes such as freezing, refrigeration ice-making, heating, evaporation, dehydration, caning processes and analyses in scientific laboratory and scientific infrastructure.

In Kenyan Vision 2030 foresees a prosperous and newly industrialised country, with medium income and high quality of life. To achieve this, we require generating capacity of 17760Mw which will assure the citizens of affordable reliable and adequate electricity supply. Currently, Kenya has per capita power capacity of 28.57w and this is grossly inadequate even for domestic consumption. Energy demand is projected to increase by 11.9% to 15.3% per annum. This would mean a n increase from current 7670GWG to 77307GWG (Kenya power statistics). This puts Kenya way below South Africa which has power capacity of 1047W and UK which has power per capita of 1266w (7). Access to reliable and stable supply of electricity is a major challenge for both the urban and rural dwellers, only about 40% of the nation's 45million has access to the grid electricity. An analysis of Kenya's electricity supply problems and prospects found out that the electricity demand at the coast of Kenya outstrips the supply and utility. Consumer outlets are crying for alternative cheap complementary power backups which stand in the gap and mitigates for hydropower which is epileptic and erratic with the trends of global warming and extended droughts. The situation is likely to be more volatile. The acute electricity supply not only hinders the country's development but also restricts socioeconomic activities to basic human needs and adversely affects the quality of life in general (Sunday Oyedepo 2014). This is contrary to the go green and go blue movement which encourages tapping into the endowment of the seasonal oceans which are locking a myriad of jobs and wealth of world population.

*(Continued on next page)*

Power disruption in Kenya negates economic performance and impedes the research industry. At KMFRI one year and half 24hour cycle of one hour interval monitoring of cut-in wind speed using an innovative cloth and balloon anemometer improvised techniques exhibited that hydropower supply is not reliable and can cause untold damages to the research industry. Put on hold, the server industry sets scientists and other stakeholders at bay for a couple of hours up to 2days. However the answer to the present imbroglio may be found in renewable and sustainable forms. Wind hybridized solar will make up for low peaks either way in this wind sunshine portfolio belt.

Research have shown that developing nations such as Nigeria, Morocco, South Africa among others are endowed with abundant energy resources which have not been properly managed to satisfy the respective nation's energy needs (2,4,8,10). Overdependence and excessive fixation on oil and gas has slowed down the development of alternative sources of energy, even when the need is glaring. In a bid to attract and encourage investment in renewable energy in Kenya, the government needs to put in place the necessary mechanisms that will aid renewable energy development and production. One of the challenges of this effort is the absence of legal framework to regulate the industry or enforcement of these regulations. Clear rules, legislative roles and responsibilities of various stakeholders along every stage of the energy flow from supply to end use are elements of the overall policy framework needed to promote renewable energy technologies such as policy legal and institutional framework are at their infancy stage in Kenya and are being developed under the reformed program (S. O. Oyedepo 2014).

Effective policy and regulatory framework for advancing renewable energy in Kenya like in Nigeria are paramount to achieve long term reduction in carbon emission, enhance the energy security in the country, promote the policy of diversifying the energy supply so as to include renewable resources and technologies to nation's energy supply mix and make electricity accessible to rural dwellers through grid extension and mean grid considering the level of electrification in the country is very low.

### Materials and Methods

The study was conducted at KMFRI premises of coordinates 40° 055'S long 39° 682'E. A questionnaire was administered to monitor power break ups for a period of 12months between 2014 and 2015. An experiment was set up to monitor wind strength and solar radiation at the creek, overlooking Fort Jesus between December 2014 and June 2016. A cloth and balloon were taken to mimic the conventional anemometer. The speed of the wind was derived from the angle subtended by the cloth. The cloths were tied on three mangrove posts, approximately 4meters tall, in a periodical sequence of two, three and four replicated thrice. The degrees subtended were derived using Bourfot Ranking Scale. Accuracy of the data was validated using the balloon method. The solar radiations were also recorded on an hourly basis, daily based on the solar insolation aspect. Other parameters taken include humidity, wind direction, tidal rhythms and the weather conditions.

### Results and Discussion

A 16 month study conducted at the Kenya coast on the assessment potential proved that the area is viable Solar radiations per day in the month of September to March resonates between 6-8 hours while the wind cut- in speed blows between a monthly mean of 11-15 ms<sup>-1</sup> which has a great potential for electricity generation.

### Interpretation

One boxplot being higher than another means that there is a difference between the months.

The Median is a measure of central tendency just like the mean. The population mean and population variance would be equal (homogeneity in variance) if the boxplots are nearly on the same levels. There is strong evidence of unequal variance (no relationship between mean and variance) although there is no evidence of consistent non-normality (skewness).

### Analysis of Variance Table

#### Response: Treatments

|               | Df | Sum Sq. | Mean Sq. | F value | Pr(>F)   |
|---------------|----|---------|----------|---------|--|
| Serial Number | 13 | 654.32  | 50.332   | 56.845  | < 2.2e-16 *** (p-value < 2.2×10 <sup>-16</sup> ) |
| Residuals     | 28 | 24.79   | 0.885    |         |  |

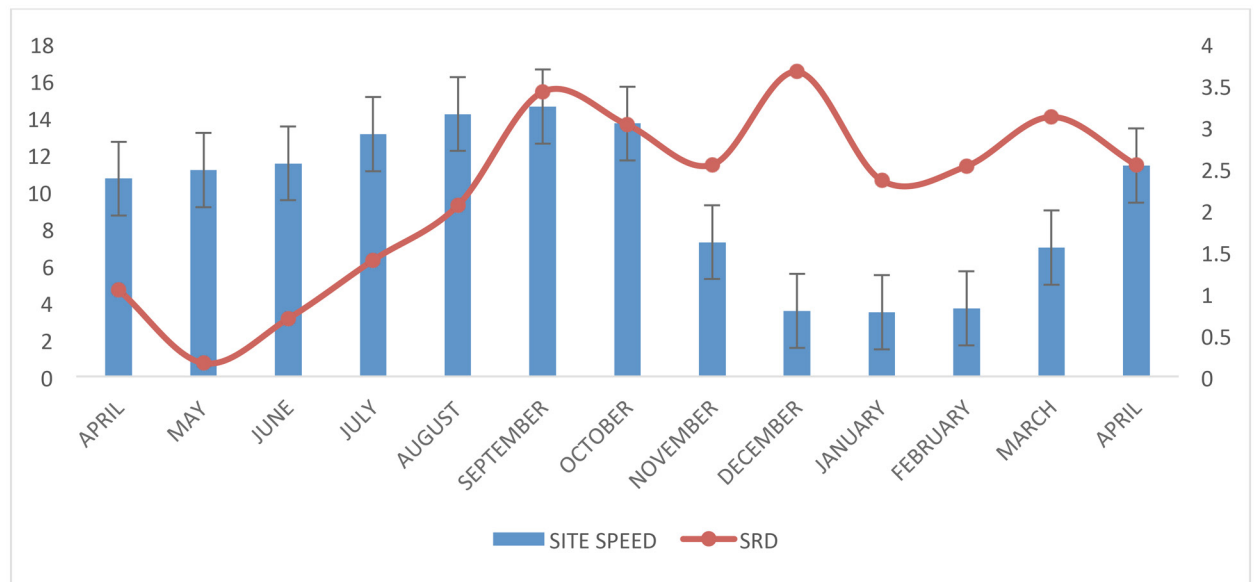
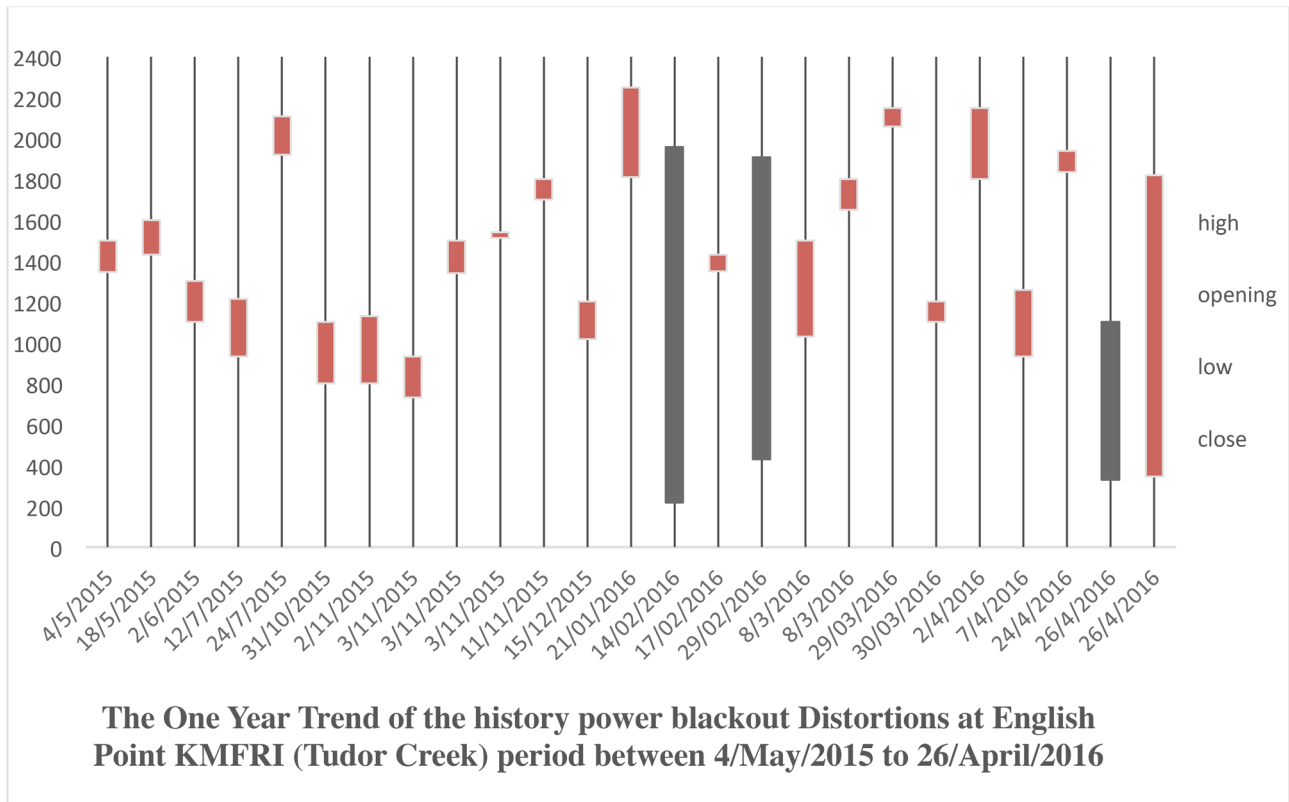
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Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

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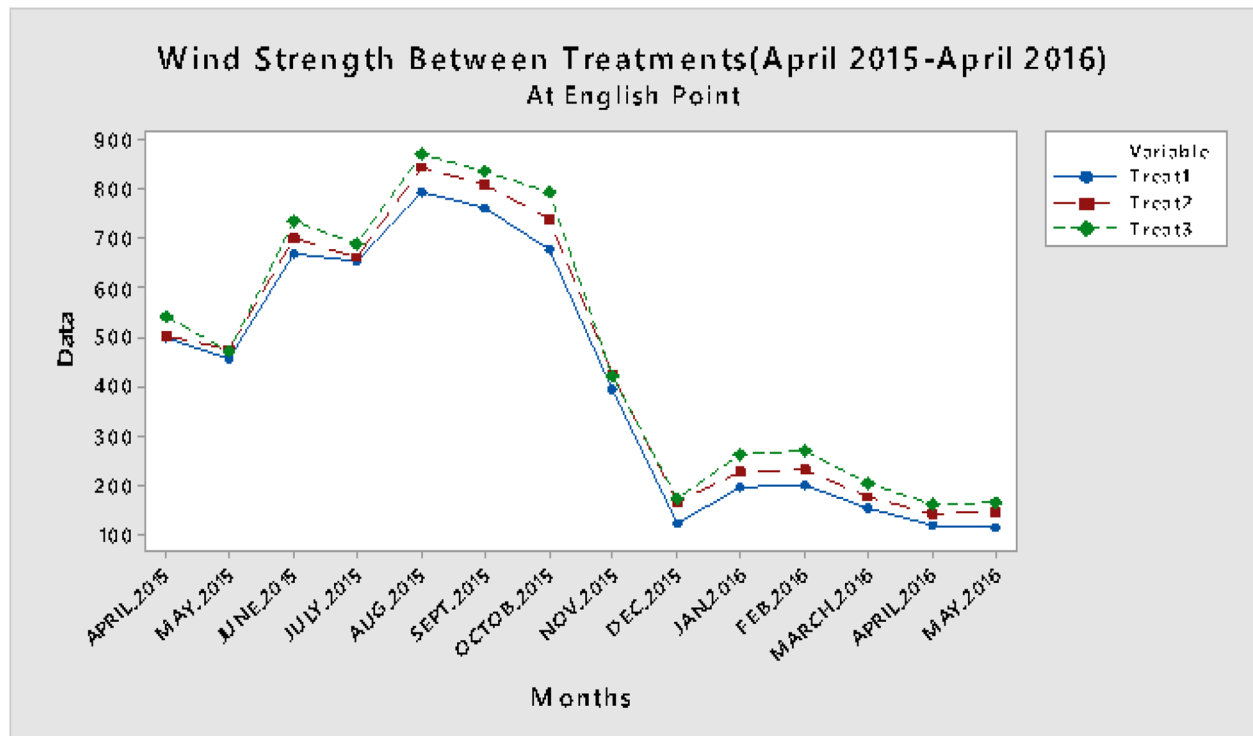


**Conclusion:** Reject the null hypothesis, there is a significant difference in the cut in wind speed during the April (2015) and May (2016) Period.



**Figure 1: Mean monthly cut-in wind speed and solar radiation dynamics at Tudor Creek, English point (2014-2016)** Source: Maroko and Mbodze 2016

(Continued on next page)



The trend of the speed(m/s) of wind at English Point between and among treatments from April 2015 to April 2016.

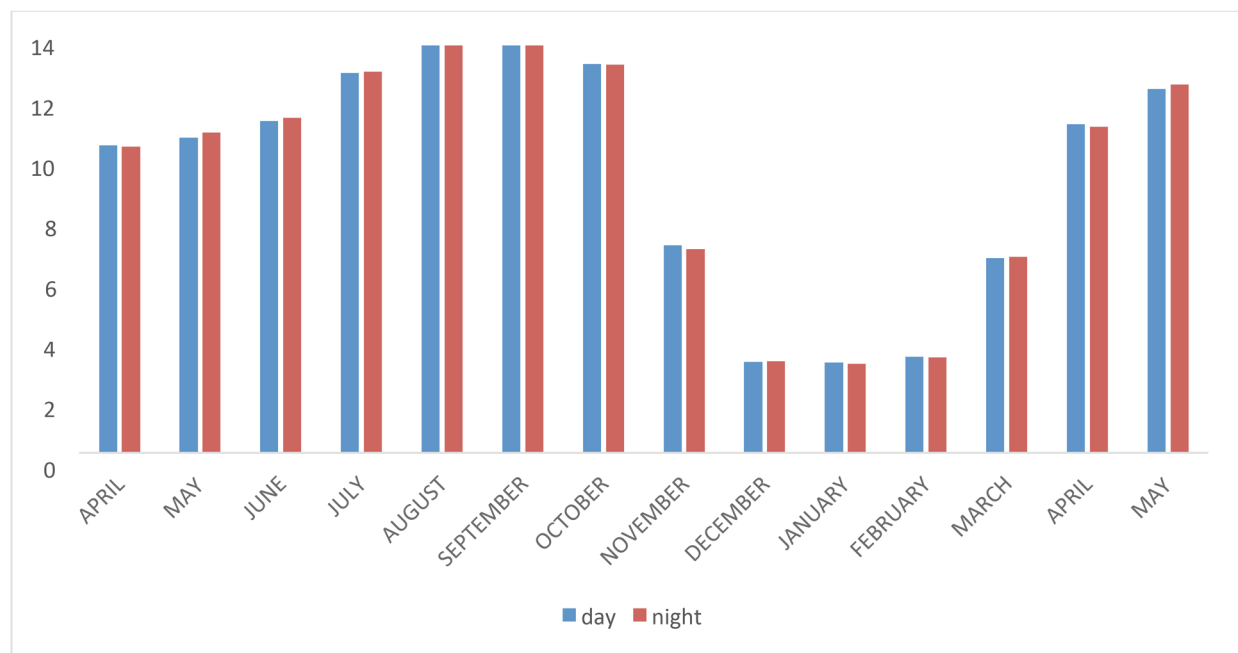


Figure 2: Mean cut-in wind speed trends scenario at day and night at English point in between April 2015 and May 2016. The figure above reveals that wind strength is relatively similar all year round. This postulates that power supply will not be intermittent.

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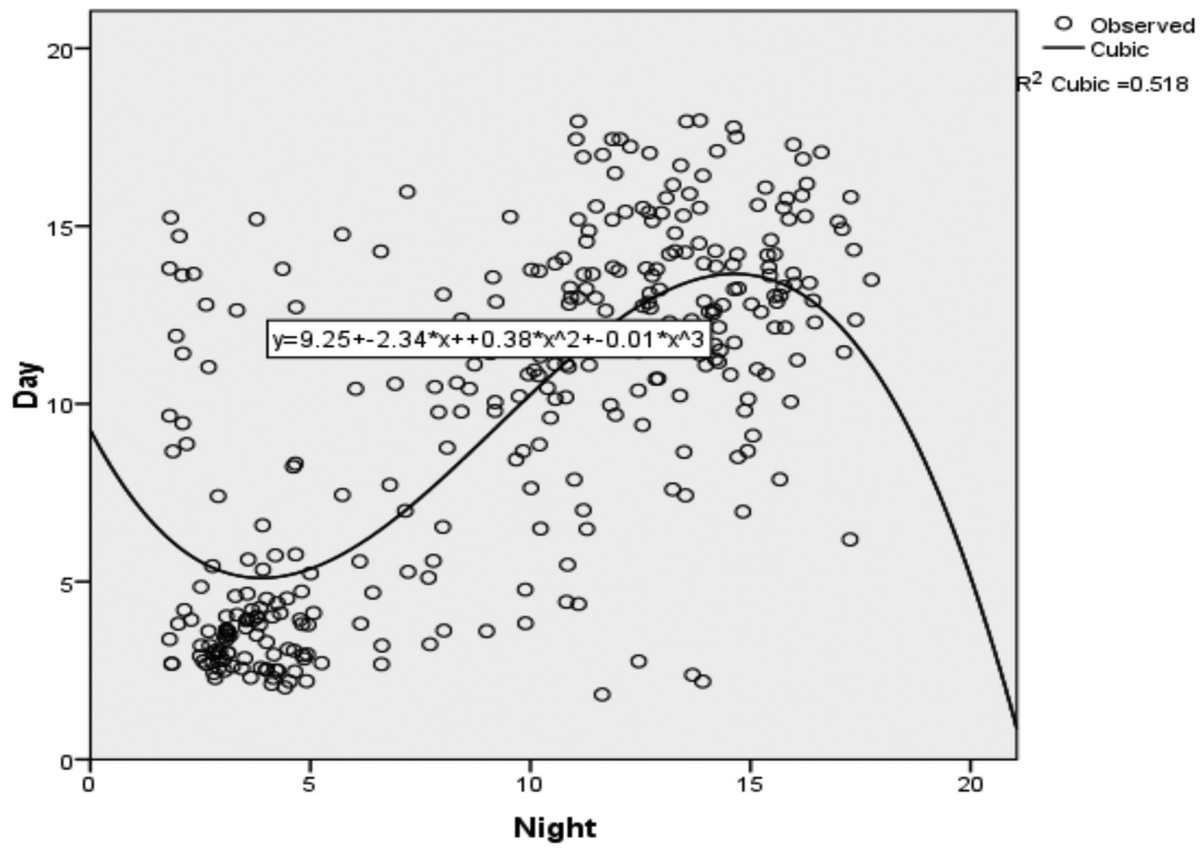


Figure 3: The cubic model reveals that the day and night are not similar in strength.

## **IMPLEMENTING A MOBILE-BASED APPLICATION FOR MARKETING AND TECHNICAL SUPPORT: DEVELOPING A SUSTAINABLE SYSTEM FOR FISH FARMERS IN UGANDA**

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Mobile phones have a rapid diffusion rate and facilitate farmers' access to information, helping increase their bargaining power, control over external events, develop new skills and grow revenues. For instance, in Tanzania the arrival of mobile phones, transformed agricultural business performance at all points by augmenting farmers' access to education and vital market information. Matuha (2015) found that fish farmers use mobile phones to access technical guidance from intermediary farmers, obtain market information, accomplish mobile banking and receiving, contact family members and make plans for procurement of fish farming inputs.

Factors that seemed to discourage mobile phone use included: lack of electricity, poor network coverage, high calling credit and maintenance costs, lack of awareness and promotion. On the other hand, information regarding stocking and harvesting, feeding management, pond construction and management, disease management, water quality management, broodstock management and market prices were information topics most needed by fish farmers.

Several different business models have emerged in efforts to provide technical support to African farmers with cell phones. Each varies in the level of public sector control, business model, cost, and flexibility. One commercial model invites farmers to subscribe to a fish-focused network of producers managed by a service provider who moderates the transactions and may be compensated by subscription fees, transaction fees, or commissions. The entrepreneur firm builds and supports a network of suppliers, producers, and buyers whose transaction costs support the network. The source of technical information may be uncertain, but the responsiveness to technical questions may be rapid because the entrepreneur is motivated to keep and grow the number of participants. This is the approach we take in Uganda.

The purpose of this paper is to describe the implementation of a mobile-based application for fish farmers, participation processes, and services provided. The conclusion considers how ICT advances food security and development by empowering farmers and linking them to each other, extension, and input suppliers.

## **MONO FEEDING OF SEA-CUCUMBER *Holothuria scabra* WITH SEAWEED AND MARINE PELLETS: A COMPARATIVE STUDY**

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Sea cucumbers are a delicacy and occupy an important part of Asian cuisine. Owing to their nutritional, biomedical and environmental benefits, aquaculture of these marine gems is gradually gaining momentum. Due to lack of information on the nutritional requirements and preferred diets, this has hindered the sustainable culture methods. However the present study is an attempt to find out the suitable and preferred diet of commercially important species *Holothuria scabra* fed with red seaweed *Kappaphycus striatum* (KS) and marine pellets (MP) under artificial condition. After 60 days of feeding trial, no significant difference was observed in their growth performance in terms of condition index, specific growth rate, length-weight relationship, feed conversion ratio and ingestion rate. The stable isotope analysis also confirmed some interesting observation. Hence the results obtained from the study provide us with an overview of the ingredients selected and therefore an additional clue to further investigate upon the optimum nutritional requirements of this species.

## IMPACT OF GOLDEN MUSSEL CONTROL ON THE COSTS OF TILAPIA BREEDING IN CAGES

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Fish farms installed in Brazilian public water reservoirs have been infested by the golden mussel, *Limnoperna fortunei* (Dunker, 1857), causing economic impacts and reducing the cage service life by up to 50%. In these areas, the mollusc finds both a substrate for attachment and, probably, food available from the organic load released by the activity. This paper aimed to evaluate the economic impact of golden mussel control on tilapia breeding in different production scales and cage volumes. Twelve fish farms were selected in the Ilha Solteira (Paraná River), Canoas II and Chavantes (Paranapanema River) reservoirs, in the State of São Paulo, Brazil. The following criteria were used for choosing the farms: 1. Golden mussel infestation in the area; 2. Production scales according to CONAMA Resolution No. 413/2009 (S=Small < 1,000 m<sup>3</sup>; M=Medium from 1,000 to 5,000 m<sup>3</sup>; and L=Large >5,000 m<sup>3</sup>); 3. Different cage volumes (6 to 18 m<sup>3</sup>); and 4. Different cage cleaning systems and structures. Information on disbursement and capital required for calculating such control costs was obtained through a semi-structured questionnaire. The expenses with labor, maintenance, and depreciation of infrastructure and equipments used in cleaning were accounted for, as well as cage maintenance and additional depreciation, and costs with energy or fuel for cleaning. The annual depreciation caused by mussel infestation was determined by the linear method. The value obtained was divided by the total cubic meters (USD m<sup>-3</sup> year). All other costs were determined by multiplying the unit amount (USD hour<sup>-1</sup>) by the number of hours required to perform the mussel control operation and the results were expressed in USD m<sup>-3</sup> year. Medium-sized enterprises had the lowest costs, regardless of the cage volume. The highest costs resulted from cage and washing machine depreciation and maintenance. Costs were higher in the 6 m<sup>3</sup> cages than in the 18 m<sup>3</sup> ones, mainly in the small fish farms (S), whose costs were twice as high, since time spent to take the cages to the washing site was similar, thus reducing labor and energy costs for the 18 m<sup>3</sup> cages.

**Table 1.** Golden mussel control costs (USD m<sup>-3</sup> year) for tilapia culture in different production scales and cage volumes, in July 2016 (USD1.00 = R\$3.278).

| Costs                                   | 6m <sup>3</sup>          |                                |                          | 18 m <sup>3</sup>        |                                |                          |
|---|--------------------------|--------------------------------|--------------------------|--------------------------|--------------------------------|--------------------------|
|   | S<br><1000m <sup>3</sup> | M<br>1000 a 5000m <sup>3</sup> | L<br>>5000m <sup>3</sup> | S<br><1000m <sup>3</sup> | M<br>1000 a 5000m <sup>3</sup> | L<br>>5000m <sup>3</sup> |
| Sampling Number                         | 3                        | 2                              | 3                        | 3                        | 4                              | 3                        |
| Annual Costs (USD m <sup>-3</sup> year) | 37.47                    | 19.37                          | 24.22                    | 18.50                    | 14.25                          | 17.77                    |
| Labor                                   | 2.79                     | 1.45                           | 3.27                     | 0.96                     | 0.80                           | 0.84                     |
| Energy or Fuel                          | 1.01                     | 0.22                           | 0.61                     | 0.32                     | 0.13                           | 0.29                     |
| Depreciation                            |                          |                                |                          |                          |                                |                          |
| Cages                                   | 8.47                     | 8.90                           | 5.08                     | 3.86                     | 5.47                           | 8.71                     |
| Washing Infrastructure                  | 1.02                     | 0.24                           | 0.73                     | 0.43                     | 0.24                           | 0.52                     |
| Washing Machine                         | 5.11                     | 0.18                           | 2.20                     | 1.87                     | 0.13                           | 0.15                     |
| Maintenance                             |                          |                                |                          |                          |                                |                          |
| Cages                                   | 9.91                     | 7.79                           | 7.08                     | 6.52                     | 7.02                           | 6.80                     |
| Washing Infrastructure                  | 1.03                     | 0.17                           | 0.82                     | 1.04                     | 0.14                           | 0.31                     |
| Washing Machine                         | 8.13                     | 0.42                           | 4.41                     | 3.50                     | 0.32                           | 0.15                     |

## **VETERINARY DRUG RESIDUES IN SEAFOOD INSPECTED BY THE US FDA –WHAT ARE THE FINDINGS**

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Seafood is the most globally consumed food commodity. It is forecasted that the world's population will require 232 million metric tons (MT) seafood by 2030, around 62 million MT more than it can be expected from wild-caught fisheries production.

In the past decades, the seafood market has been continuously changing and shifting from wild-caught to farmed fish. Aquaculture has been increasing on average at 7% per year since 1970. In 2014, its contribution to the supply of seafood for human consumption surpassed that of capture fisheries. However, this race to achieve a higher aquaculture production level might compromise on food safety and quality.

Infectious diseases in aquaculture are prevalent and required a proper management strategy. Veterinary intervention such as vaccines and pharmaceutical products, including antibiotics, are used for prevention and treatment of diseases. The application of proper treatments can be one of the effective management tools. But, their inappropriate use can lead to problems related to increased frequency of bacterial resistance and the potential transfer of resistance genes from the aquatic environment to other bacteria. Furthermore, imprudent use of veterinary drugs may also result in the occurrence of their residues in aquaculture products. Consequently, regulatory agencies of importing countries responsible for inspection of seafood products can pose import restrictions in order to mitigate consumer exposure to drug residues that may pose health risk.

This presentation will discuss trends in veterinary drug violations based on data reported by US FDA inspections over the past years.



## GREENHOUSE BASIL AND KALE PRODUCTION USING RAS EFFLUENT AND SOLIDS IN SOUTHERN AUSTRALIA

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There is a growing need for aquaculturalist to reuse waste streams to reduce operating costs and improve environmental outcomes. Horticulture can exploit solid and liquid wastes produced in recirculating aquaculture systems (RAS) due to their high nutrient content relative to less intensive production systems. Leafy greens plant species are well suited to the nutrient composition of aquaculture waste streams, as seen in aquaponics systems. Their production using a combination of liquid and solid waste is assessed in this research.

A 7m<sup>3</sup> pilot-scale RAS was stocked with jade perch (*Scortum barcoo*) at a density of 30kg.m<sup>-3</sup> and maintained at a feeding rate of 1% fresh weight per day. Solids were removed from the RAS through filtration (150 µm screen) and sedimentation (HRT = 0.5hr). Settled solids in the sedimentation tank were removed daily and sludge was thickened to 10% dry weight.

Basil (*Ocimum basilicum*) and kale (*Brassica oleracea* var. *acephala*) seedlings were transplanted to individual pots, filled with a sandy-loam soil, and drip-irrigated inside a pilot-scale greenhouse for six weeks (from 11 September until 23 October 2015). The six treatments were designed to test the horticultural benefit of RAS effluent compared to fresh water as well as the value in applying dried sludge vs wet sludge. Wet sludge was collected over a period of weeks. The dry sludge treatment was prepared by dewatering sludge through a 150 µm filter and air drying sludge on black polyethylene inside the greenhouse. The treatments were; T1 – effluent with dry sludge; T2 – effluent with wet sludge; T3 – fresh water only (control); T4 – effluent only (control); T5 – fresh water with wet sludge; T6 – fresh water with dry sludge.

Results showed that when irrigated with RAS effluent, the addition of wet and dried sludge to basil and kale plants in soil significantly improved fresh weight production. When combined with fresh water for irrigation, dried sludge also improved fresh weight gain for both plant species, however, wet sludge did not. Soil samples for dry sludge treatments showed a high concentration of plant available phosphorus compared to wet sludge samples among other more favourable plant growing conditions. The result of the research confirm the benefits of reusing RAS effluent for plant production and present a case for the dewatering and air drying of RAS sludge, compared to direct sludge application, to further improve plant growth and nutrient recovery.

| Treatment | Fresh Weight Gain   |                       |
|-----------|---------------------|-----------------------|
|           | Kale                | Basil                 |
|           | [g]                 | [g]                   |
| T1        | 35.8 ± 3.7 <b>a</b> | 36.4 ± 3.72 <b>a</b>  |
| T2        | 22.1 ± 7.9*         | 34.8 ± 3.10 <b>ba</b> |
| T3        | 10.8 ± 0.5 <b>d</b> | 28.8 ± 10.82*         |
| T4        | 29.5 ± 2.3 <b>b</b> | 26.9 ± 6.43 <b>dc</b> |
| T5        | 10.9 ± 1.7 <b>d</b> | 19.7 ± 0.94 <b>e</b>  |
| T6        | 22.7 ± 2.3 <b>c</b> | 27.2 ± 1.48 <b>c</b>  |

\*Unreliable results due to replicate variation

## CONTRIBUTION OF NATURAL FEEDING AND INERT DIET FOR AMAZON RIVER PRAWN REARED IN DIFFERENT SYSTEMS USING STABLE ISOTOPE ANALYSIS

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Amazon River prawn (*Macrobrachium amazonicum*) is omnivorous species and adapt very well to different farming systems. The aim of this study was determine the relative contributions of food sources in Amazon River prawn farmed in different systems using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  composition. The treatments were: freshwater prawn monoculture (PRAWN), Integrated Multi-trophic Aquaculture with tambaqui and free prawn (IMTA-FREE), Integrated Multi-trophic Aquaculture with tambaqui in cage and free prawn (IMTA-CAGE). Samples of consumers and food sources such plankton, organic matter in sediment, fish faeces, periphyton and inert diet were collected during the culture to determine  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  composition.

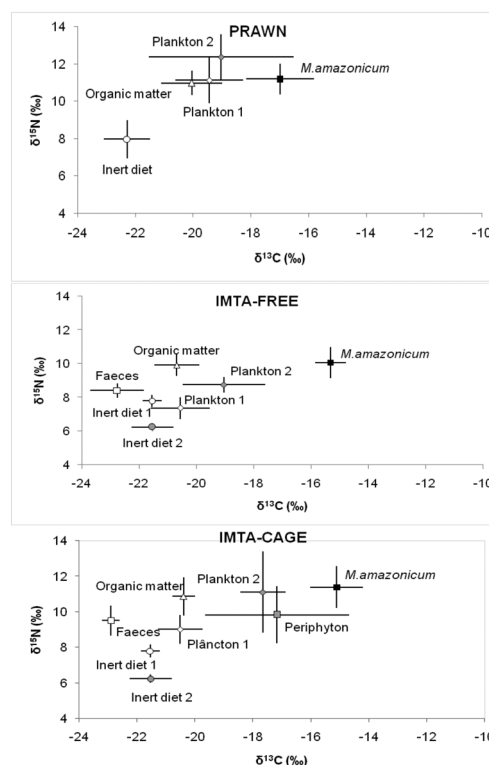
By the stable isotopes composition, was applied a Bayesian Mixture Model to estimate nutrient sources contribution in freshwater prawns to determine the trophic position of consumers in different farming systems.

In addition, were determined the growth performance and water quality in all treatments. The results showed that there were differences in prawn muscle and nutrient sources ( $P \leq 0.05$ ) between treatments.

Amazon River prawn does not depend on only inert diet (~40%), but also on autochthonous sources (~60%) inside the ponds. In IMTA-FREE and IMTA-CAGE systems, part of organic matter produced by tambaqui such as fish faeces (~16%) and waste fish feed (~40%), supplemented prawns feeding in these farming systems.

For this reason, the results suggest that this specie has a great potential to be co-cultivated in IMTA systems. Thus, the farmed species could be an excellent alternative for a more sustainable aquaculture.

(CNPq/CAPES/FAPESP)



**Figure 1.**  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  composition in Amazon River Prawn farmed and their food sources.

## APPLICATION OF DUAL STABLE ISOTOPE ANALYSIS TO ESTIMATE THE NUTRITIONAL CONTRIBUTION OF FISH MEAL AND MICROALGAL BIOMASS TO THE GROWTH OF PACIFIC WHITE SHRIMP

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The microalgal biomass represents one of the emerging groups of microbial-derived ingredients that have been increasingly tested and applied in the aquaculture feed industry. The present study aimed to evaluate the nutritional contribution of the dietary nitrogen, carbon and total dry matter supplied by fish meal (FM) and microalgal biomass of *Spirulina* (SP) and *Nannochloropsis oculata* (NO) to the growth of Pacific white shrimp *Litopenaeus vannamei*. Carbon and nitrogen stable isotope analyses were done to the different ingredients, experimental feeds and shrimp samples. Seven isoproteic (36 % crude protein), isoenergetic (4.7 Kcal gr<sup>-1</sup>) experimental diets were formulated. Three diets consisted in isotopic controls manufactured with only one main ingredient supplying dietary nitrogen: 100% FM (diet 100F), 100% SP (diet 100S) and 100% NO (diet 100N). Four more diets were formulated with varying proportions of these three ingredients: 33% of each ingredient on a dietary nitrogen basis (diet 33FSN) and the other three included a 50:25:25 permuted proportion for each of the three ingredients (diets 50FSN, 50SFN and 50NFS).

At the end of the bioassay, shrimp fed on the three control diets showed significantly different weight gains. Diets formulated with the three main ingredients elicited similar weight gain, except diet containing 50% of NO. Isotopic analysis indicated that 1) The microalgal biomass substituting FM was rapidly incorporated in all dietary treatments and shrimps reached full isotopic equilibrium with their respective diets in 3 weeks, 2) Proportions of incorporated dietary nitrogen in shrimp bodies (observed values) were significantly different to those established in the formulated diets (expected values). Higher proportions of dietary nitrogen and dietary carbon were supplied by FM and SP. In contrast, the biomass derived from NO contributed significantly lower proportions of nutrients to shrimp tissue accretion. Results highlight the nutritional suitability of *Spirulina* as FM replacement ingredient. Although microbial-derived proteins are still relatively expensive, new production technologies allow predicting lower prices and a wider range of application of these alternative ingredients.

Table 1. Weight gain, survival rates and estimated proportions of nutrients supplied by fish meal and microalgal biomass of *Spirulina* and *Nannochloropsis oculata* to the growth of Pacific white shrimp *Litopenaeus vannamei*

|                 | Diet |      |      |         |          |          |          |
|-----------------|------|------|------|---------|----------|----------|----------|
|                 | 100F | 100S | 100N | 50FSN   | 50SFN    | 50NFS    | 33FSN    |
| Weight gain (%) | 451  | 300  | 121  | 314     | 308      | 232      | 275      |
| Survival (%)    | 80   | 92   | 90   | 85      | 83       | 93       | 90       |
| RDC (%)         |      |      |      |         |          |          |          |
| Nitrogen        | -    | -    | -    | 53-43-4 | 53-34-13 | 32-24-44 | 35-50-15 |
| Carbon          | -    | -    | -    | 46-47-7 | 55-29-16 | 37-19-44 | 29-52-19 |
| Bulk dry matter | -    | -    | -    | 52-41-7 | 50-33-17 | 40-21-39 | 33-47-20 |

RDC: Relative dietary contributions in relation to proportions of dietary inclusions in each experimental diet, either 50:25:25 or 33:33:33

## IMPACT OF IMPROVED SYSTEMS ON RICE AND COMMON CARP *Cyprinus carpio* MULTITROPHIC TROPICAL AQUACULTURE, ANTANANARIVO, MADAGASCAR

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Flooded rice fields are ecosystems favorable to the growth and production of many aquatic organisms. They can even play a major role in the feeding and nutrition of local communities, as a source of self-recruiting species or by supporting the production of farmed fish in association with rice. In Madagascar, integrated rice-fish aquaculture systems are a 150+ years-old tradition. The initial species was goldfish *Carassius auratus*, locally known as *Trondro gasy* (meaning, “the Malagasy fish”) but now, the common carp (*Cyprinus carpio*) took over as the main produced species, sometimes in polyculture with tilapia. Although traditional, the technology has recently been improved by several organizations (FAO, APDRA) by building larger and higher side dykes and digging a canal in the middle of the field, which serves as a refuge area for fish when the field is drained. However, this also implies an approximate 10% loss of space for rice production, a basic food commodity for local populations, which is supposedly compensated by higher rice productivity in integrated system.

The first aim of this farm experiment was therefore to assess the productivity of these systems by characterizing the importance of the different trophic compartments in traditional (rice + self recruiting species) and integrated systems (rice+carps improved systems, Fig. 1). However, nutrient availability can be a limiting factor, as feed and fertilizers are expensive. Trophic deadlocks can then be suspected in iron-rich areas due to phosphorous chelation in sediments. As common carp is a detritivore/omnivore fast-growing species which trophic behavior (bioturbation) involves re-suspending sediments, it is expected that its introduction in inundated rice field will improve nutrient availability and increase rice and fish production. The second scientific question is then focused on determining the nutrient pathways and deadlocks, and how they impact the common carp, rice and fish production.

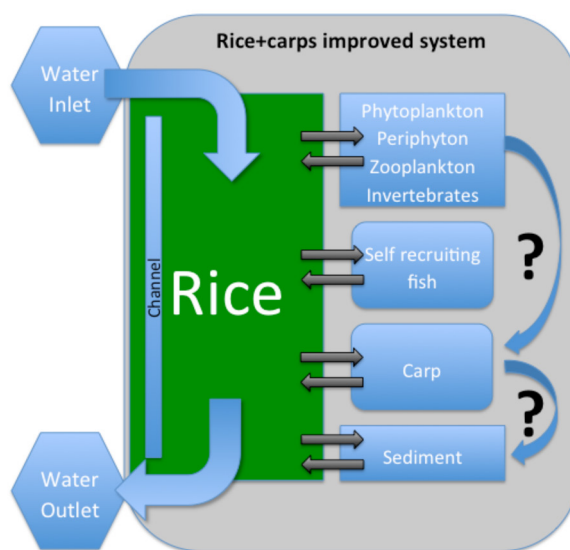


Fig 1: Experimental design of “rice+carps improved system”

## INTEGRATED FISH FARMING SYSTEMS IN MADAGASCAR: THE TANJONA FROM FENOARIVO

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The Fenoarivo City in the vicinity of Antananarivo, Madagascar, is surrounded by floodplains where a new kind of fish farming named “Tanjona” appeared since ten years. Tanjona refers locally to large earth’s dyke raised in the middle of a floodplain to realize different kind of culture (e.g. vegetables, rice, fruits; Fig. 1). Within this study the word “Tanjona” is used for a broader meaning including also an integrated fish farming system and even livestock (e.g. ducks, cattle). The aim of this study was to allow a first description of this system, realizing a census of all Tanjona, a description of technical pathways, and an analysis of production factors and of the agrarian and social system.

Nine Diagnostic surveys were realized and 27 Tanjona were counted with production cycles of 6 to 12 Months. Two large typologies of fish farming systems are commonly observed in these Tanjona: those with fish farming in ponds and culture on the dykes and those with integrated rice-fish aquaculture with either culture on dykes. Fish farming occurs usually from October to May from fish caught in the neighboring Lake Sisaony or from rice fields. Yield is between 400 and 10 000 kg of fish / ha / cycle (7 to 9 months). The main reared species are tilapia (sold by weight of 100 to 500 g), common carp (sold by weight of 500 to 1 000 g) and gold fish (sold by weight of 100 to 200 g).

Tanjona may account for a new opportunity to intensify fish production in Antananarivo, considering the high yield recorded. However, floodplains from Fenoarivo also receive large amount of wastewater outflowing from Antananarivo. Therefore, prior to promote fish farming in Tanjona systems, water and fish quality characterization will need to be done in these systems.



Fig. 1: Tanjona from Fenoarivo (2016)

## CHARACTERIZATION OF FRESHWATER FISH QUALITY IN THE TANJONA SURROUNDING ANTANANARIVO: UTILIZATION OF TRACE METALS AND STABLE ISOTOPES ( $\delta^{13}\text{C}$ & $\delta^{15}\text{N}$ )

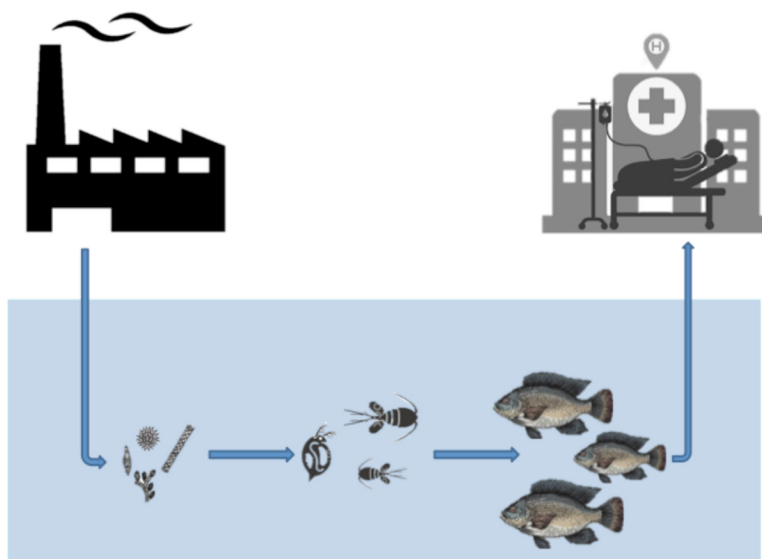
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The need for food and freshwater fish increased these last years in Antananarivo, Madagascar, with growing population. To meet these needs, several projects were founded, as the AMPIANA project that aims to feed the population of Antananarivo with fish. Of these, a new kind of fish farming named “Tanjona” appeared since ten years in floodplains of the Fenoarivo City, in the surrounding of Antananarivo. However, urbanization and population growth may affect water quality in these floodplains, and as a consequence health quality of fish reared in these systems.

Particulate and dissolved materials discharged through sewage water are incorporated in organisms at the base of the food web (Fig. 1). Contaminants from sewage water may then be transferred to aquatic ecosystems and organisms such as fish. Fish as tilapia will then constitute a source of contaminant for human health. Tilapia is an opportunistic fish that tolerates a wide range of water quality, and will be then considered in this study as an indicator for contamination in water systems.

The aim of this study was therefore to characterize the level of water contamination in the Tanjona. Trace metal concentrations in white muscle of tilapia were therefore analyzed to characterize fish quality. Also stable isotopes ( $\delta^{13}\text{C}$  &  $\delta^{15}\text{N}$ ) from fish white muscle, particulate organic matter and sediments were used to characterize anthropogenic contamination of wastewaters. Results from Tanjona were then compared with one water system considered as contaminated (Masay Swamp) and one non contaminated (Andranotapahina Lake).



g. 1: Schematic pathway of trace metals contamination



# BIOGENIC ACIDIFICATION REDUCES SEA URCHIN GONAD GROWTH AND INCREASES THE SUSCEPTIBILITY OF AQUACULTURE TO OCEAN ACIDIFICATION

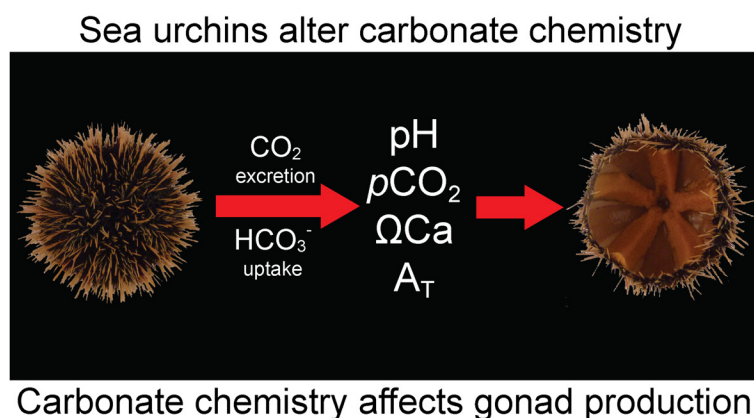
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The changes in the ocean caused by uptake of  $\text{CO}_2$  (ocean acidification) has emphasised the importance of seawater carbonate chemistry on growth of calcifying marine organisms. However, calcifiers can also change the carbonate chemistry of surrounding seawater through respiration and calcification, a potential limitation for the aquaculture of these organisms.

This study examined how seawater exchange rate and density of the sea urchin *Tripneustes gratilla* affects the carbonate chemistry of their culture water and in turn drives variation in growth and gonad production. Growth, relative spine length, gonad production and consumption rates were reduced by up to 67% by increased density (9 to 43 individuals.m<sup>-2</sup>) and reduced exchange rates (3.0 to 0.3 exchanges.hr<sup>-1</sup>), but survival and food conversion efficiency were unaffected. Analysis of the influence of seawater parameters indicated that reduced pH and calcite saturation state ( $\Omega\text{Ca}$ ) were the primary factors limiting gonad production and growth. Uptake of bicarbonate and release of respiratory  $\text{CO}_2$  by *T. gratilla* changed the carbonate chemistry of surrounding water. Importantly total alkalinity ( $A_T$ ) was reduced, likely due to calcification by the urchins. Low  $A_T$  limits the capacity of culture water to buffer against acidification.

This study demonstrates the importance of seawater carbonate chemistry and biogenic acidification in inhibiting the productivity of calcifiers in culture. Furthermore, it highlights the vulnerability of aquaculture to predicted increases in atmospheric  $\text{CO}_2$  concentrations and acidification of source water, which are likely to exacerbate the changes in carbonate chemistry in culture waters of marine calcifiers due to reduced buffering capacity. Direct management to counter biogenic acidification will be required to maintain productivity and reproductive output of marine calcifiers.





## **COPING WITH EFFECTS OF CLIMATE CHANGE-THE CASE OF SEAWEED FARMERS IN ZANZIBAR, TANZANIA**

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Seaweed farming in Zanzibar, Tanzania has been carried out for close to thirty years now. It has proved to be a reliable economic activity for the rural population especially women. However, in the past ten years, farmers are facing challenges that are related to climate change, factors that have negatively affected their lucrative economic activity. Such factors are increase in surface seawater temperature which has caused failure to cultivate the higher valued seaweed (*Kappaphycus*) in the current farming sites in shallow water areas, as well as bleaching leading to ice-ice disease that cause mortalities of the farmed seaweed. Other effects include severe erosion especially on the East Coast and the ever increasing Zanzibar population which bring additional nutrients to the ocean causing algal blooms that have also caused mortalities of both the higher valued *Kappaphycus* and the lower valued and hardy *Eucheuma*.

Innovative farming methods have been developed to help cultivate the higher valued seaweed in deep waters of 2-6m depth at low tide. These methods include floating rafts made of nylon ropes and bamboo which faced challenges of breakage and loss of the seaweed. Recently, a new method called tubular nets, a method used in other countries, has been experimented with better results. These efforts show that the higher valued seaweed can be farmed in deeper waters. There is need to pilot and expand the tubular nets method for increased production of the higher valued seaweed.

## USING SAP FROM THE SEAWEED *Eucheuma denticulatum* AS PLANT GROWTH STIMULATOR FOR ENHANCED FOOD PRODUCTION IN TANZANIA

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Despite the many years of farming seaweed in Tanzania, value addition leading to production of high end industrial products has not started. Equally, production of value-added products remains very low and still based on home-based products such as soaps and foods whose market is still a challenge. Value addition such as the use of seaweed sap as plant growth stimulator to enhance food production has been recommended in Tanzania and elsewhere.

An experiment is being conducted to study the use of seaweed sap as plant growth stimulator on three garden food crops. The seaweed *Eucheuma denticulatum* was collected from the farms and transported to the laboratory. The juice (sap) was extracted from the wet seaweed by using kitchen juicer, and stored in the laboratory. Seeds of three food crops were purchased from agricultural shops. Soil was placed in four growth containers and the seeds were planted in the four containers each making four separate setups. In the first set-up, the sap was diluted with freshwater at a ratio of 1:1 (sap/water) and used to irrigate the seeds. In setup 2, the ratio was 1:0.5 and in the third setup the sap was not diluted. A control was also set where no sap was added. Three replicates for each setup were made. The seeds were irrigated with tap water every two days and the sap was added once per week. Preliminary results show that the plants that received undiluted sap had the least growth whereas the best growth was in the ratio with 1:1 sap and water.

## **COLLABORATION BETWEEN A FARMER INITIATIVE AND AN NGO GIVES A BRIGHT FUTURE FOR SEAWEED FARMERS IN ZANZIBAR, TANZANIA**

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Milele Zanzibar Foundation (MZF) is an NGO that aims at improving the living standards of coastal communities through education, health and livelihood programmes. In 2015, MZF started working closely with the Zanzibar Seaweed Cluster Initiative (ZaSCI) to improve livelihoods of seaweed farmers especially women who are the majority. ZaSCI is a Triple Helix initiative that links seaweed farmers and small-scale processors to research institutions, the government and financial institutions/organisations. Starting its activities in 2006, ZaSCI has seen much improvement in the members' economics especially women who are the majority through innovative seaweed farming and value addition. MZF has enabled ZaSCI to increase the number of seaweed value-added products from 50 to 70 in one year, compared to 2 products before the start of ZaSCI.

Additionally, MZF has enabled ZaSCI members to hold quarterly meetings that have resulted into holding the First Zanzibar Seaweed Day on 23<sup>rd</sup> July 2016. The seaweed day brought together more than 800 people including members of ZaSCI, other seaweed farmers, fishermen, government officers (from local to central government), NGOs, and the general public. The Revolutionary Government of Zanzibar (RGoZ) took this Seaweed Day to be a National Day to be celebrated every year on 23<sup>rd</sup> July. Likewise, RGoZ stated that it will provide 500 seaweed harvesting badges (small boats) to farmers. More people in Zanzibar and Tanzania in general knowing that seaweed can be used in the country rather than continuing exporting as raw material to multinational companies. The number of people eating seaweed has increased. ZaSCI and MZF will hold the 2<sup>nd</sup> Seaweed Day this year on 23<sup>rd</sup> July 2017 targeting the urban community. MZF will also conduct trainings to ZaSCI members and other seaweed farmers in aspects of entrepreneurship, financing, business development, and marketing (so far MZF has reached about 1,000 farmers).

It is expected that the number of people knowing about using seaweed and actually eating seaweed will double, and the number of seaweed products will increase by a third by 2018, one year after the 2<sup>nd</sup> seaweed day and the internal market of seaweed will expand.

## CHALLENGES ASSOCIATED WITH PhD. SUPERVISION IN SUB-SAHARAN AFRICA

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Research project supervision plays a paramount role in timely completion and outstanding quality of PhD. Thesis. Effective supervision is a conglomerate of various artifacts built around exceptional skills, capacity building as well as mutual understanding of supervisee/ supervisor.

A systematic literature review was conducted on the supervisee/supervisor relationship in sub-Saharan Africa. Literature was searched were using the keywords: “supervisor”, “Supervisee”, “PhD” and “Thesis” in combination with “Sub-Saharan Africa”. These searches were supplemented by reviews of lists for relevant publications in peer reviewed scientific journals or other media. The recovered information were included based on their regarding supervisor/supervise challenges and relationship.

Findings showed that there are a number of challenges that are faced in the supervisory process. Among them are negative attitude by the supervisor which are aggravated by student uncoperativeness, laziness, gender, origin as well as unclear rights and obligations. Besides students may become aggressive on to their supervisors due to untimely commenting on their submitted work due to either tight programs by the supervisors, negligence or insufficient knowledge in the subject area. This in many cases ends up in sour relationship that leads to untimely completion of the studies by the students.

The findings and suggested solutions to this study will from enhance an excellent relationships between supervisor and supervisee that will bring effective supervision leading to timely completion and excellent research output and theses.

## COMPARATIVE GROWTH PERFORMANCE OF *Oreochromis niloticus*, *Clarias gariepinus* AND *Cyprinus carpio* AT A HIGH ALTITUDE ENVIRONMENT

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The Kenya government policy on Aquaculture Economic Stimulus Program (ESP) led to increased culture of warm water fish species in all parts of the country including the high altitude areas. There was an unprecedented increased culture of Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*) and common carp (*Cyprinus carpio*) in the Mount Kenya region. However, performance of these species in such altitudes has rarely been documented. This study evaluated the growth performance of the three species in the high altitude areas near Mt. Kenya region. Fingerlings, with a mean weight of 4.9-5.6g, were stocked in earthen ponds at 3.3 fish m<sup>-2</sup>. Feeding was done twice a day at the recommended body weight. Water quality and growth performance and was recorded daily and every two months respectively. After 420 days of culture period, final mean weight of *C. gariepinus* was 785.4±8.6g representing a mean weight gain of 780.4±5.4g and SGR of 2.9%. *O. niloticus* recorded a final mean weight was 148.1±3.2g, which translated to 143.1±2.8g in weight gain and SGR of 2.2% while *Cyprinus carpio* weighed 328.2±6.8g which translated to 323.2±4.6g in terms of weight gain and SGR of 2.5%. Survival was 68%, 96%, and 95% for *C. gariepinus*, *O. niloticus* and *C. carpio* respectively. There was no significant difference in water quality parameters in all ponds under trials and they were within tolerance levels for the culture period. Relatively low temperature in this study (13–18°C) was still suitable for culturing warm water species at the high altitude and indication that these species can acclimatize to high altitude and still grow well.

## EFFECTS OF ANTHROPOGENIC POLLUTION WITH SPECIAL REFERENCE TO HEAVY METAL ACCUMULATION IN BIVALVE MOLLUSKS (*Saccostrea cucullata*) IN SUNDERBAN WETLANDS OF WEST BENGAL, INDIA

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The Sunderbans (area 9630 sq km; Lat. 21° 32' and 22° 40'; Long. 88° 10' and 89° E), the estuarine delta of the Ganges–Brahmaputra Rivers, are a unique bioclimatic zone in the Bay of Bengal coast of eastern India. The heavy metals in the brackish water system generally gets deposited on the sediment bed or remain in dissolved state in the water column, depending on the nature of chemical species, which are influenced by factors like salinity, pH etc. The Hooghly–Matla estuarine complex of West Bengal is the most polluted coastal zone as this zone is presently stressed with unplanned mushrooming of shrimp culture units, industries, and hotels, which in most cases, release their respective wastes without any adequate treatment. Every animal which directly or indirectly exposed to environmental pollution develop its own natural detoxification mechanism to get rid of foreign particles entering the body. The toxicants induce their effects first at cellular or even at molecular level, but ultimately it leads to the physiological, pathological and biochemical disorders which may even cause death (Tort *et al.*, 1996). Heavy metals have ecological significance due to their toxicity and cumulative behavior by entering into the food chain. Translocation of heavy metals from water to soil to plant to fish feed and then to fish may cause bioaccumulation of these substances which in turn cause severe health hazards in human beings (Kaviraj, 1989; Mathew and Jahageerder, 1999). Benthic fish and shellfish accumulate more metals than the surface dwellers. Moreover, benthic organisms concentrate more Cu and Pb while surface feeders accumulate Zn in excess. With long term exposure, these metals tend to accumulate with the age of organism especially in the liver, gills and kidney (Maiti and Banerjee, 2007). Shellfish, especially oysters are used as biomonitor organisms worldwide because of their ubiquity, sessile way of life, filtering mode of feeding and relatively long shelf life of metals in their body. The current study looks into the variations of heavy metal concentrations in the soft tissue of oyster (*Saccostrea cucullata*) commonly found in Sunderban Island. The impact of relevant physicochemical parameters (like surface water temperature, pH and salinity) on the metal accumulation levels of the ambient aquatic phase has also been assessed in the present programme as these variables have considerable effect on the fate and speciation of heavy metals in the estuarine system. The edible oyster *Saccostrea cucullata* collected from each of the seven sampling stations along with surface water and sediments during pre monsoon (Mar–Jun'08) and monsoon season (Jul–Oct'08). The concentrations of heavy metals in the aquatic phase and surface sediment (biologically available heavy metals) were analyzed using Atomic Absorption Spectrophotometer (Perkin Elmer: Model 3030) as per the standard procedure. The results of this study indicate that concentration of Zn, Cu were significantly higher in the oyster tissue than in the sediments. However, concentration of Pb was lower compared to levels in the sediment. This suggests that *Saccostrea cocullata* has more affinity for some metals such as Cu and Zn. More details are discussed in the paper. ent.

## FIRST DETECTION OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS IN FARMED RAINBOW TROUT IN KENYA

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Rainbow trout (*Oncorhynchus mykiss*) was introduced in Kenyan rivers in 1920s primarily for sport fishing. However, it later gained economic importance with farmers establishing commercial farms for rearing the fish. The Kenyan government set up a hatchery to supply fry and fingerlings to farmers in 1948. Over the years, increased demand for stock from farmers has led to importation of fertilized eggs from Europe and America. Importation of breeding materials has potential to facilitate pathogen transfer.

Infectious hematopoietic necrosis (IHN) is a notifiable fish disease that has not previously been reported in Kenya. The objective of the present study was to screen some farms in Nyeri county following reports of mortalities.

Eight farms were visited and fish observed in the ponds. Those manifesting ill-health and healthy looking fish were collected for tissue sampling (n=20 per farm). Samples of headkidney, stored in RNAlater® were used for extraction of RNA which was then used to synthesize cDNA. Polymerase chain reaction (PCR) of the samples yielded a product of about 680 bp. Sequencing of this product confirmed for the first time, the presence of IHN virus in rainbow trout in Kenya.



## THE EFFECTS OF ALGAL FOOD QUALITY ON BEHAVIORAL AND MORPHOLOGICAL GUT STRUCTURE OF ORNAMENTAL FISH (*Tropheus brichardi*) IN LAKE TANGANYIKA

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Anthropogenic changes in primary producer quality due to reductions in nutrient loading and increases in terrigenous sediments may alter the nutritional value of attached algae at the base of the food chain. Animals may compensate for low food quality by becoming more selective, increasing ingestion rate, or through compensatory changes in gut morphology. Optimal foraging theory predicts that animals will modify their behavior to maximize energy and nutrients intake per unit time.

We assessed the influence of periphyton quantity and nutritional quality on the amount of time devoted to feeding, the duration of feeding bouts and the feeding rate (bites/sec) during an individual feeding event of fish (*T.brighardi*) in Lake Tanganyika using a linear regression model. We also assessed the effect of food quality on relative intestine length, stomach fullness and condition factor. Our results reveal that algivores respond to variation in food quality through both behavioral and morphological modifications. Algivores fed selectively on diatoms, which are rich in essential fatty acids (EFA) at all sites, but fish at sites with poor food quality (low %P) spent less time feeding in each bout. There was a positive correlation between sediment accumulation on the periphyton and both gut length and stomach fullness. The increase in relative intestinal length may facilitate a more complete extraction of nutrients and energy.

Our result also reveals high fish density in high quality sites tends to increase the feeding pressure, this may affect algal biomass. However, the combined effects of lower algivore densities and lower feeding rates along a gradient of sediment accumulation led to a decrease in grazing pressure at high sediment sites. Sediment covers and dilutes algae, thus deplete the algal quality. This may increase the digestion costs to herbivores, and consequently, produces a negative cascading effect through the food chain.

## STUDY TO MAP AQUATIC ANIMAL DISEASES IN NORTH AFRICA

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As a preliminary step to developing a continental biosecurity programme for the prevention of transboundary diseases, the InterAfrican Bureau for Animal Resources of the African Union (AU-IBAR) initiated a mapping study of aquatic animal diseases in the five regions of Africa. This presentation describes the results of a study carried out during late winter and early spring, 2016, in the countries of North Africa including Algeria, Egypt, Libya, Mauritania, Tunisia and Western Sahara.

The study comprised an initial review of peer-reviewed publications. The results of the literature search informed the drafting of a questionnaire which was circulated to 76 individuals in the six countries including the OIE Focal Point and/or Chief Veterinary Officer, Chief Fisheries Officers, the senior executive in a number of fisheries NGOs as well as the first author of any relevant paper indentified during the literature review. The questionnaire contained a range of questions on the infrastructure associated with disease diagnosis, surveillance and control, national and on-farm biosecurity practices, aquatic animal health status including disease outbreaks and associated risk determinants, undergraduate veterinary education and continuing professional development training courses in fish medicine and disease.

The response was disappointing with only five completed questionnaires returned. The final report on the diseases present in North Africa was drafted using information in the returned questionnaires and a review of fish diseases in Egypt written by Professor Aly (2013) for CGIAR. The majority of diseases reported were caused by opportunistic pathogens that are endemic in most countries where Tilapia, catfish, carp, mullet, seabass and sea bream are reared. Only limited information was provided on the role of veterinarians/veterinary paraprofessionals and the laboratory capacity in the region to service the aquaculture industry.

As a consequence of the various mapping studies AU-IBAR organised two workshops in Cairo in March 2017. The first workshop dealt mainly with the diagnosis and prevention of fish diseases. The second one was a consultative workshop to develop regional aquatic animal disease control frameworks. At the end of the consultative process all the delegates from North Africa, which also included Sudanese representatives agreed to work towards developing a disease control framework along the lines of the guiding principles used by the Southern African Development Community (SADC).

## **ADDRESSING GENDER-BASED CONSTRAINTS TO LIVELIHOODS OF MALE AND FEMALE INFORMAL FISH RETAILERS IN EGYPT**

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This paper offers recommendations for researching and addressing gender inequalities in informal fish markets, based on field-tested methodologies, the development of key indicators and analysis of results from longitudinal studies of gender-responsive market interventions. From 2012 to 2016, under the WorldFish Project 'Improving Employment and Incomes through the Development of Egypt's Aquaculture Sector', this research implemented two gender-sensitive surveys of informal fish retailers in five governorates in Egypt. Following initial assessments of gender-based constraints to retailer livelihoods, market interventions were planned using gender-transformative approaches and implemented in five retail market areas. Market interventions implemented among beneficiary groups provided support in the form of financial capital (establishment of VSLA groups), physical capital (distribution of ice boxes and tricycles), human capital (provision of finance & accounting training), and social capital (formation of retailer committees and theatre action groups) to women retailers. Following these interventions, impact assessments were conducted to compare gendered outcomes of fish value chain developments between beneficiary and non-beneficiary groups of women retailers. Results indicate increases in gross income, improvements in net profits and diversification of markets enterprises among beneficiary retailers. Baseline and endline surveys also indicate changes towards more gender-equitable attitudes and women's empowerment in terms of decision-making within beneficiary households. This provides the first longitudinal study of gender-based constraints and gender-responsive market interventions in Africa's fish value chains. From these assessments, it also provides analysis of the economic significance of gender-based constraints to retailer profitability in Egyptian contexts. Final recommendations call for further research to develop value chain empowerment index, in order to evaluate the significance of gender-based constraints in varying informal market contexts. Further research is also needed to explore links between equitable shifts in household gender roles and increases in women retailer's profitability.

## **SHELLFISH FARMING IN NAMIBIA: A SUCCESS STORY FROM SOUTHERN AFRICA**

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Namibian mariculture is currently expanding significantly, primarily because international markets for oysters are being opened up, particularly in Asia. Up till now Namibia's main mariculture species has been Pacific oysters, and its main market South Africa. Only having one key market stifled production and Namibia's mariculture industry remained small until recently. Oyster in Namibia are being breed and grown since the 80's, by the Klein's family. Since 2007 it was observed an increase in the number of active companies producing oyster, as this is the leading seawater farming activity in Namibia. Besides farming oysters, Namibia mariculture is characterized by farming seaweed, abalone and mussels. In 2007, there were 13 food business operators (FBO) located between Lüderitz (2); Walvisbay (8) and Swakopmund (3), and more than 30 permit holders to produce a variety of seawater aquaculture products. In 2009, there were only 1 FBO in Lüderitz, 2 in Swakopmund and 3 in Walvisbay

The Oyster industry started making international buyer contacts in South East Asia, through business trips, as a foundation for starting their production operations around the beginning of 2006. They obtained such large international orders that several companies had to cooperate in an attempt to meet demand. There is a real opportunity to expand production for other Namibian oyster companies by establishing good market contacts internationally. This involves market research, visits to international trade shows, and visits to international clients to cement relationships and meet client product specification requirements. There are two main farming regions in Namibia, the Karas region where Lüderitz is located and the Erongo region, where Walvisbay and Swakopmund are located. Lüderitz oyster farming activity is characterized by longline system located in the lagoon. In Walvisbay, the oyster farming occurs in the long-line systems in the lagoon and at the Walvisbay salt pans in pole/raft system. In Swakopmund, the oyster farming happens at the Swakopmund salt pans with poles/rafts and is where the hatchery is located. Besides the oysters, the abalone is farmed in Lüderitz on flow-through land-base system, and the mussels are harvest from suspended ropes located in Walvisbay lagoon.

The two main grow-out areas in Namibia are the Lüderitz and the Walvisbay lagoon. These systems have different environmental conditions. Looking at the phytoplankton distribution, Lüderitz lagoon is characterized by diatoms species (81.0%) and Walvisbay lagoon present a seasonality of diatoms and dinoflagellates, with an overall yearly proportion of 43.5% and 48.0%. Moreover, the Walvisbay lagoon phytoplankton is up to 10x more than in Lüderitz. This difference in concentration provides business threats and opportunities for the farming of shellfish at both locations. The events of algae with the capacity to produce Diarrheic Shellfish toxin are more frequent in Lüderitz. However, the levels recorded for DSP in shellfish are generally below the safety level for human consumption. In Walvisbay the low oxygen events are more frequent, either because of algae blooms or due to sulphide events.

The oyster farming activity in Namibia reached a stable delivery capacity to human consumption of almost 5 million oysters and an on-growing capacity of 10 million. The local hatchery has the capacity to produce 48 million spats/year, and the growing areas are still not being used at its maximum carrying capacity. The production capacity is being limited by the access to market, with the Republic of China and Hong Kong being the critical key players for the sustainability of the FBO. The South African market is also a crucial for the sustainability of the farming activity in Namibia, not only because it is an important market for oysters for direct human consumption, but it is also pivotal for the on-growing Namibian oyster market. The sustainability of the industry is being hindered by the limited access to markets. Markets such as EU, USA, Russia and other Asian markets are stricter in their import regulations. Their recognition of the top quality of the Namibian oyster is an asset that is not being fully used. To get access to them and make use of the Namibian quality recognition, it is required that the Namibian oysters consistently comply with approved standards. The Namibian Shellfish Monitoring and Sanitation Program is a critical tool that is being finalized by the Ministry of Fisheries and Marine Resources and the Namibian Standard Institute and in cooperation with FBO. This tool should provide the confidence to the FBO in getting access to new markets, ensuring the business long term sustainability.

## AQUACULTURE AND AQUARIA – THE SYNERGIES, SYMBIOSIS AND SUCCESSES

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Although rooted in the same technologies, aquaculture and public aquaria are not usually considered in the same context. Aquaculture involves the cultivation of aquatic organisms under controlled conditions, the purpose of which is to produce a sustainable source of protein as an alternative to wild capture fisheries. Aquaria are seen as the aquatic counterparts of zoos, housing live species for public view. In recent years the purpose of many aquaria has evolved to have conservation, education and even research goals, and this evolution provides the opportunity for the roles of aquaria and aquaculture to intersect. Both industries must focus on the health of their animals to produce a good product. Aquaria focus on creating a healthy environment in which their animals are content enough to engage in natural behaviour, such as courtship and breeding. This has led to aquaria-based breeding programs, designed to encourage genetic diversity among captive populations and relieve the pressure on natural populations as sources of new animals for exhibition. Likewise, a vital aspect of aquaculture is the production of quality seed stock through hatchery-based breeding programmes. In South Africa, this convergence has been recognised and incorporated as a component in the development of the local marine finfish industry. In fact, South Africa's first commercial foray into the culture of a local marine finfish, the dusky kob *Argyrosomus japonicus*, was a direct result of the collaboration between Cape Town's Two Oceans Aquarium, where fertilised eggs were collected, and the Department of Agriculture, Forestry and Fisheries' Research Aquarium down the road, where the eggs were hatched and the first larval rearing protocols developed. Subsequently, South Africa's other major public aquarium, uShaka Sea World, has played a significant supporting role in the development of the dusky kob industry along the east coast of South Africa. This presentation will describe the roles played by these aquaria in the development of the South African marine finfish industry to date; identify the synergies between aquaculture and aquaria in general; and discuss the future role of public aquaria in aquaculture in the South African context.

## **OPTIMISING AQUARIUM FEEDING STRATEGIES BY UTILISING AQUACULTURE FEEDING PRACTICES AND DEVELOPMENTS**

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Creating optimal environmental conditions and offering good quality nutrition to stock are both strong focus areas within aquaculture production and public aquaria. For aquaculture, this should contribute to promoting growth and ensuring top quality final product, whereas in public aquaria it should contribute to exhibiting healthy looking display specimens.

Aquarium animals often live longer and grow bigger compared to wild animals due to being housed in controlled environments, without active predation and therefore reduced stress levels. However, these fish also often present fatty livers, obesity and various other secondary health issues, due to the daily supply of unbalanced diets.

Utilising formulated feeds can contribute to meeting specific nutritional requirements of animals in public aquaria. The aquaculture industry has developed a multitude of different formulations for core cultured species. Public aquaria generally house thousands of fish of hundreds of different species, whereas aquaculture facilities hold millions of fish, usually of a single species, but of various cohorts and thus also with different nutritional needs.

Aquaria could match species specific nutritional needs to the large variety of formulated feeds already developed and can so have access to more affordable and higher volumes of formulated feeds compared to that available to private fishkeepers in pet shops. Aquaria can therefore focus on developing lean nutritional plans that support immune functionality and stress reduction, enhancing display qualities such as colour and satisfying feeding responses, all characteristics incorporated in existing formulated aquaculture feeds. Converting feeding practices in public aquaria from mainly feeding fresh fish to using formulated feeds could reduce feed costs, waste production and dependency on fisheries production which will contribute to improved sustainability. Incorporating feeding practices used in the aquaculture industry can contribute to improved feeding strategies in public aquaria.

## POTENTIAL USE OF BIOREMEDIATION METHODS FOR AQUACULTURE WASTES IN COASTAL AREAS

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Currently, aquaculture is considered as one of the fastest growing food-production industries in the world due to practically stagnant and declining fisheries, higher global demand for fish due to increased human population, change in diet preferences from red meat to healthier among others. In the effort to meet this increasing demand, production systems have been progressively intensified. Despite their high output, the intensive culture practices have adverse and negative impact on the environment. They are associated with both uncontrolled use of feed and massive production of waste which if released into the environment untreated, it deteriorates the water quality, leads to eutrophication, causes and spreads parasite and diseases and causes metals and antibiotics pollution. Other impacts include destruction and alteration of natural habitats, depletion of wild stocks, salinization of adjacent soils and change of biodiversity. These risks and impacts from aquaculture has often being criticized by environmental organisations, politicians and has generated great scientific interests and great advocacy for undertaking mitigation measures. In Spain, the aquaculture industry is widely distributed in Southern coastal areas, whereby they produce high negative environmental impacts. Bioremediation techniques which are widely known to be environment friendly, healthy, efficient and cost-effective methods for improving the quality of aquaculture waste have been proven to reduce environmental damage. In addition, bioremediation techniques in aquaculture are easy to implement and maintain, can be performed on-site and/or off-site, and reduce the amount of waste to be landfilled. In the present work, we describe the bioremediation strategies that could be applied for ameliorating negative impacts of the aquaculture effluents in coastal area towards sustainable aquaculture. This was achieved by reviewing various bioremediation concepts and strategies used in various parts of the world. Then, describing major aquaculture facilities within coastal areas and their associated environmental impacts. Finally the study carries out comparative reviews of potential bioremediation strategies which can be applied in the aquaculture waste treatment in Coastal areas.



## POST-GRADUATE TRAINING AND RESEARCH IN AQUATIC AND ENVIRONMENTAL HEALTH IN EASTERN AND SOUTHERN AFRICA

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In Eastern and Southern Africa there is a documented knowledge gap at higher educational institutions related to fish health and environmental stressors. This includes lack of understanding of aetiologies, diagnostics and prophylaxis of fish diseases. Lack of knowledge hampers the economic development of aquaculture and fisheries industries in the region, precludes evidence-based policies and decision-making from governments and also has societal implication for the development of rural areas. There is also a need to build competence in fish and environmental health to monitor and protect wild freshwater and marine fish populations which are essential for food security and safety in the region.

To address these challenges, a NORAD funded project entitled “Capacity Building for Training and Research in Aquatic and Environmental Health in Eastern and Southern Africa (TRAHESA)” was initiated in 2014. The project is coordinated in the South by Sokoine University of Agriculture in Morogoro, Tanzania and in the North, by the Norwegian University of Life Sciences in Oslo, Norway. Other participating institutions are University of Nairobi, Kenya, Makerere University, Uganda, Institute of Marine Science, Zanzibar and University of Zambia.

The project’s overall objective is to train trainers in partner institutions in the field of health of aquatic animal resources through PhD and Msc training. A secondary objective is to establish an African centre for research in the health of aquatic animals to meet research challenges that face the aquaculture industry in the region.

Our approach was to recruit 10 PhD students, 2 each from the five participating institutions in Africa. Their studies are ongoing and they are all approaching the end of their studies. Next, we established a two-year regional Msc program in the health of aquatic animal resources at SUA with initially 10 students. Teaching staff in the beginning included Professors from the Norwegian Veterinary School but also with the help of local and regional experts. A second intake of another 13 students was admitted last year with participants from Kenya, Uganda, Zambia and Tanzania. Finally, an African center for the training and research of aquatic animal resources has been established at SUA.

## OCCURRENCE AND LEVELS OF POPS IN MARINE FARMED AND WILD FISH FROM TANZANIA, A PILOT STUDY

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Aquaculture as an important food production system is a fast-growing industry in Tanzania. A number of communities especially in the coast rely on fish and fishery products from the wild and more recently mariculture for their livelihood and income generation. Milkfish (*Chanos chanos*) and mullet (*Mugil cephalus*) are omnivorous fish species, which are suitable for mariculture and preferred for food by some of the coastal communities. The rapid growing population, urban developments and agricultural activities have potential input of harmful chemicals including Persistent Organic Pollutants (POPs) in the marine environment. These chemicals have been documented to cause health effect in several aquatic organisms including fish and human beings.

The main objective of this study is to investigate the occurrence and levels of POPs in marine farmed and wild fish from Tanzania

In 2016, farmed and wild milkfish and mullet from the coastal area in southern Tanzania (Mtwara) and Zanzibar islands (Pemba and Unguja) were collected and analysed for POPs, including organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and perfluorinated compounds (PFAS). *p-p'*-DDE was the major pollutant with the highest levels in Mtwara wild milkfish (56.57 ng/g ww) (Fig 1). It was 450 times higher than the highest level in farmed milkfish from a nearby pond (0.12 ng/g ww). The high ratios of *p-p'*-DDE/*p-p'*-DDT in milkfish and mullet from Mtwara and Pemba wild indicate the historic use of DDT in the environment while the low ratio in milkfish from Shakani, Jozani and Mtwara pond indicate recent use. The PCB pattern was dominated by PCB 153>180>138. BDE 47 was the dominating congener, and was found in highest levels (0.13 ng/g ww) in the milkfish from Shakani on Unguja. Other POPs were in low levels. PCBs and BFRs originate from human activities and urban emissions. In general, the POPs levels in the current study are lower than the MRLs of the EU and USFDA, implying that the fish are safe for human consumption but may pose ecological and fish health risk and threaten biodiversity. However, the findings from the present study warrants further research on POPs on other aquaculture operations.

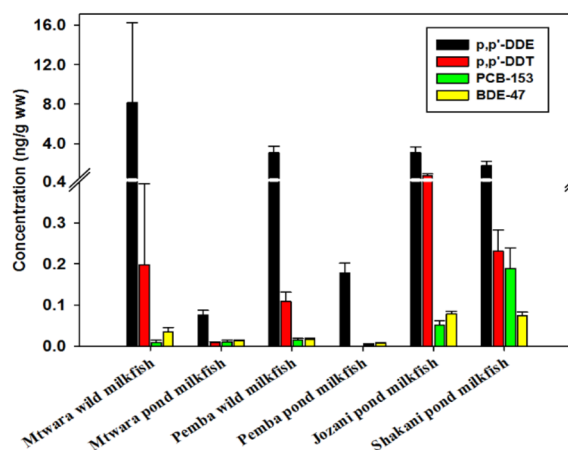


Figure1: Concentrations of dominant congeners in the different sites

**TITLE OF PAPER: GROWTH AND SURVIVAL OF MUD CRAB, SCYLLA SERRATA, REARED IN TWO CULTURE SYSTEMS WITHIN MIDA CREEK MANGROVES, COASTAL KENYA**

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The growth and survival rates for mixed sex mud crabs was investigated for a period of 230 days using bottom and floating cages, and two feed types in a crab fattening experiment. Crabs attained harvestable weight by the third month for both floating ( $466.2 \pm 137$  g) and bottom cages ( $542.2 \pm 109.3$  g). There was no significant difference in mean total weight of mixed-sex crabs in the two culture systems ( $t = 1.75$ ,  $p > 0.05$ ), however males showed significantly higher total weight gain compared to females. A higher overall survival rate was found for crabs cultured in floating cages (63.8%), compared to bottom cages (44.9%). There was no significant difference in growth performance between crabs fed gastropod tissues and those fed fish offal. The long term specific growth rate for floating cages (0.69 g/d) was not significantly different from that of bottom cages (0.92 g/d). An analysis of potential return on investment showed the floating cages to be more profitable per production cycle compared to the bottom ones. The floating cage system and mono-sex male crab culture are recommended to farmers within mangrove tidal flats in Kenya.

## SEDIMENT NUTRIENT CONCENTRATION AND PREVALENCE OF ENDO AND ECTO-PARASITES IN RELATION TO *Oreochromis niloticus* STOCKING DENSITY IN EARTHEN POND CAGE CULTURE

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Ammonia and phosphorus concentrations above natural levels and parasitic infections together have been recognized as one of the detrimental and limiting factors in fish productivity in aquaculture. The present study determined ammonia, phosphorus and organic matter in the sediments and parasitic occurrence in 100 *Oreochromis niloticus* using parasitological examination procedures. The sediments for nutrient analysis and fish samples for parasite identification were collected from two sets of cage; the first set (S1) with 400 fingerling and the second set (S2) with 800 fingerling of *O. niloticus*. The third set (S3) for nutrient concentration only was outside the cage. The results indicated that ammonia and phosphorus were highest ( $4.54 \pm 0.11 \mu\text{g/L}$ ) and ( $0.92 \pm 0.04 \mu\text{g/L}$ ) respectively in S2 and lowest in S3 ( $1.84 \pm 0.08 \mu\text{g/L}$ ) and  $0.18 \pm 0.03 \mu\text{g/L}$ . Percentage organic matter content was highest in S2 ( $11.7 \pm 0.17\%$ ) and lowest in S3 ( $6.02 \pm 0.30\%$ ).

A total of 1 protozoan, 5 helminthes and 2 crustacean groups were identified. Most parasites were more abundant in cages stocked with 800 fingerling (S2) compared to cages with 400 fingerlings (S1). The common groups of parasites were Digenean helminthic such as Contracaecum species, Clinostomums species and blackspot metacecaria. The results suggest that stocking density had influence on organic matter and nutrients concentration. The presence of some digenean helminthic parasites such as contracaecum species, clinostomums species, and blackspot metacecaria call a need for control strategies for parasites to be undertaken.

## EFFECTS OF FREQUENCY OF GRADING ON THE GROWTH, INTRA-COHORT CANNIBALISM AND ECONOMIC BENEFITS OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*, BURCHELL, 1822) CULTURE

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The profitability of *C. gariepinus* still suffers from poor growth performance, low survival and high cases of cannibalism. Attempt that aims at increasing economic benefits must therefore target strategies that decouple link between catfish culture with poor growth survival and cannibalism. This study determined the effects of in-pond grading frequency on the growth performance, cannibalism and economic benefits of *C. gariepinus* in tank culture system. Three treatments designated as G0 (no grading), G2 (grading every 2 weeks) and G4 (grading every four weeks) in a completely randomized design (CRD) was applied in triplicate. The tanks were stocked with 200 fingerlings each. Sampling was conducted weekly to measure length and weight of fish. Mortalities were recorded and dead fish observed under dissecting microscope to ascertain that it is due to cannibalism. An enterprise budget was conducted to determine the economic benefits of grading frequency. Data were analyzed using One-way ANOVA. Growth performance in terms of final mean weight, weight gain, Specific Growth Rate (SGR) and Food Conversion Ratio (FCR) were affected by frequency of fish grading. The *C. gariepinus* graded every two weeks grew better than those graded every four weeks and were all above the no grading treatment. Changes in fish heterogeneity was observed after day 42 and continued until day 60. The size variation was significantly affected by grading frequency. The mean TL of fish graded every 2 weeks was the highest, followed by grading every four weeks and finally the no grading treatment was the lowest. Fish size heterogeneity (CV% and skewness) was consistently the highest in *C. gariepinus* in no grading treatment followed by fish where grading was done every four weeks while it was lowest in treatment where grading was done every two weeks. Mortality owing to cannibalism was affected by grading frequency where highest cannibalism mortality occurred in *C. gariepinus* where there was no grading followed by grading every four weeks and intermediate at grading frequency every four weeks. Meanwhile other mortality by other causes such as wounds and suffocation in fish did not differ with grading frequency. Highest total fish yield, net returns above TVC and TC was obtained in treatment where grading was done every 2 weeks. The enterprise budget analysis of grading frequency in the present study indicated that it is economically feasible to culture *C. gariepinus* when grading was done every four weeks but the best economic returns occur when the grading frequency is done every two weeks.

## **ISSUES TO CONSIDER IN DEVELOPING AN EFFECTIVE REGIONAL COLLABORATIVE FIT-FOR-PURPOSE PHD PROGRAMME IN FISHERIES AND AQUACULTURE**

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With the socio-economic transformation and ensuring food security being the focus in sub-Saharan African countries, the need for trained and well skilled labour force to drive this process has become even more apparent. More doctorate holders will be required to provide improved management and innovative solutions to these countries' challenges. Efforts by individual countries to produce doctorates have been curtailed by limited resources both human and infrastructure, especially in regards to research based doctoral programmes. Their higher education curricula require urgent revision to improve the quality of degrees and to make them more relevant to respective national development agendas in respect to the role and contribution of the fisheries and aquaculture sectors in the region. Novel and specialised fields needed to generate appropriate solutions to Fisheries and Aquaculture development challenges in the region are currently not offered in nearly all sub-Saharan universities and other higher institutions of learning in the region, yet most countries in sub-Saharan Africa have identified these sectors as crucial for increased food security and improved livelihoods of their populations. Currently, of the few experts on the sub-Saharan region most of them attained their doctoral studies outside the region at a cost considered rather too high in the region. Resorting to development of regional collaborative doctoral programmes with international partnerships shall reduce the burden on individual countries by pooling resources together for a common cause. The process should begin with identification of suitable host of the programme followed by revision or development of appropriate curricula and identification of private sector/industries for internships and collaborative research. This should be followed by identification and re-tooling of human resources first from within the region and where necessary from outside, recruiting of students and lastly training the students. A number of issues need to be considered in this process including – education policies, broadening skills and improving gender balance, making the education transdisciplinary and participatory learning and improvement in teaching and learning infrastructure. These efforts should result into increased and better trained doctorate holders who are critical and innovative thinkers, better and effective managers that will tackle the region's challenges.

## ISOLATION AND CHARACTERIZATION OF FLAVOBACTERIA FROM NILE TILAPIA IN TANZANIA

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Fish farming is a rapidly growing sector in Tanzania, but is faced with several constraints including fish diseases. This study was conducted to identify the occurrence of *Flavobacterium specie* in Nile Tilapia (*Oreochromis niloticus*). Live farmed (n = 187) and wild Nile tilapia (n = 108) were collected from 12 ponds in Morogoro region and Lake Victoria respectively. The samples of gills, liver and kidney swab were collected for Flavobacteria isolation and characterization on the basis of colony morphology, biochemical tests, partial sequencing of 16SrRNA gene and phylogenetic analysis. A total of 69 yellow pigmented colonies were identified as flavobacterium species on the basis of colony morphology and biochemical tests and were selected for PCR amplification of the 16SrRNA gene. Fifty purified PCR products were sequenced. Of the 50 sequences, only forty clean sequences were analyzed individually by BLAST search to obtain its closest reference sequence available in GenBank.

Homology search analysis revealed that, fourty isolates were similar (97-100%) with the genus *Flavobacterium* and *Chryseobacterium*. Phylogenetic analysis grouped isolates into two different clades. Of the 40 isolates, three isolates grouped with *Flavobacterium aquaticum*, two isolates each grouped with *Flavobacterium indicum* and *Flavobacterium oryzae*. Eleven isolates grouped with *Chryseobacterium indologenes*, six with *Chryseobacterium joostei* and 16 isolates were similar to *Chryseobacterium species*. All isolates were previously reported from fish as opportunistic pathogens. This study reports the first isolation of Flavobacteria in farmed Tilapia in Tanzania.

**Table 1:** Summary of the fish carried isolates, Isolates selected for PCR and organs involved in this study

| Sampling sites     | Number of Fish with isolates | Isolates Selected for PCR | Occurrence of Flavobacteria species % |             |             |
|--------------------|------------------------------|---------------------------|---------------------------------------|-------------|-------------|
|                    |                              |                           | Gills                                 | Liver       | Kidney swab |
| Lake Victoria      | 21                           | 23                        | 56.5                                  | 34.8        | 8.7         |
| Dakawa             | 9                            | 11                        | 36.4                                  | 45.5        | 18.8        |
| Mkindo             | 6                            | 7                         | 71.4                                  | 14.3        | 14.3        |
| Mgeta Langali      | 11                           | 12                        | 58.3                                  | 33.3        | 8.3         |
| Tangeni            | 14                           | 16                        | 50.0                                  | 25.0        | 25.0        |
| <b>Grand Total</b> | <b>61</b>                    | <b>69</b>                 | <b>53.6</b>                           | <b>31.9</b> | <b>14.5</b> |

**Table 2:** Homology among the isolated strains and reference strains in the GenBank based on 16S ribosomal RNA gene, partial sequence

| Number of bacterial Isolates | Homology species in the GeneBank Database | Accession Number | Nucleotide Identity (%) With EZ- Taxon |
|------------------------------|---|------------------|--|
| Three                        | <i>Flavobacterium aquaticum</i>           | HE995762         | 98                                     |
| Two                          | <i>Flavobacterium indicum</i>             | AY904351         | 98                                     |
| Two                          | <i>Chryseobacterium oryzae</i>            | HE997061         | 98                                     |
| Six                          | <i>Chryseobacterium joostei</i>           | KU058436         | 97                                     |
| Eleven                       | <i>Chryseobacterium indologenes</i>       | KC189901         | 99                                     |
|                              |   | KF017580         |  |
| Sixteen                      | <i>Chryseobacterium species</i>           |                  | 97-99                                  |



## ISOLATION AND CHARACTERIZATION OF *FLAVOBACTERIACEAE* FROM NILE TILAPIA IN TANZANIA

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Fish farming is a rapidly growing sector in Tanzania, but is faced with several constraints including fish diseases. This study was conducted to identify the occurrence of *Flavobacterium*-like species in Nile Tilapia (*Oreochromis niloticus*). Live farmed (n = 187) and wild Nile tilapia (n = 108) were collected from 12 ponds in the Morogoro region and Lake Victoria respectively. Samples of gills, liver and kidney swab were collected and *Flavobacterium*-like isolates characterized on the basis of colony morphology, biochemical tests, partial sequencing of 16S rRNA gene and phylogenetic analysis. A total of 69 yellow pigmented colonies were identified as *Flavobacterium* related species. Of the 50 isolates subjected to 16S rRNA gene sequencing only forty good quality sequences were obtained and analysed individually by BLAST search to obtain its closest reference sequence available in GenBank.

Homology search analysis revealed that, forty isolates were similar (97-100%) with the genera *Flavobacterium* and *Chryseobacterium*. Phylogenetic analysis grouped isolates into two different clades. Of the 40 isolates, three isolates grouped with *Flavobacterium aquatile*, two isolates each grouped with *Flavobacterium indicum* and *Flavobacterium oryzae*. Eleven isolates grouped with *Chryseobacterium indologenes*, six with *Chryseobacterium joostei* and 16 isolates were similar to *Chryseobacterium* species. All isolates were previously reported from fish as opportunistic pathogens. The findings obtained in this study will help in the control of diseases caused by members of the family *Flavobacteriaceae* in future. This study reports the first isolation of members of the *Flavobacteriaceae* in farmed Tilapia in Tanzania.

Table 1: Summary of the fish carried isolates, Isolates selected for PCR and organs involved in this study

| Sampling sites     | Number of Fish with isolates | Isolates Selected for PCR | Occurrence of <i>Flavobacterium</i> species % |             |             |
|--------------------|------------------------------|---------------------------|---|-------------|-------------|
|                    |                              |                           | Gills   | Liver       | Kidney swab |
| Lake Victoria      | 21                           | 23                        | 56.5  | 34.8        | 8.7         |
| Dakawa             | 9                            | 11                        | 36.4  | 45.5        | 18.8        |
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| Sixteen                      | <i>Chryseobacterium</i> species           |                      | 97-99                                  |

## FEEDING PREFERENCES OF THE CALANOID COPEPOD *Pseudodiaptomus hessei* UNDER CULTURE CONDITIONS

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In the natural environment, feeding of estuarine copepods generally involves a diel vertical migration. It has been proposed that copepods migrate upwards at night to feed, returning to deeper strata at dawn. However, some research has shown that copepods might also be feeding on benthic food source such as detritus and microalgae during the day. This study aims to improve our understanding of feeding biology of *Pseudodiaptomus hessei*, particularly whether *P. hessei* it prefers feeding on suspended phytoplankton or benthic microalgae. Copepods were allowed to fed on two species of microalgae (*Isochrysis galbana* and *Tetraselmis suecica*), one presented as benthic and the other as a suspended food source. The choice of algae and presentation state were also swapped. The amount of each algae species in the gut of *P. hessei* was quantified using real-time PCR. Food source consumption was significantly different ( $p < 0.026$ ) between benthic and planktonic food source suggesting that *P. hessei* prefers to feed on the planktonic food source. After 80 min, *P. hessei* then shifts from planktonic to a benthic food source. The results of this study confirm the assumption from previous ecology research that *P. hessei* might be feeding on a benthic food source during the day.

## DEVELOPING SMALL SCALE COMMERCIAL AQUACULTURE IN SOUTHERN MALAWI

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The purpose of this study was to investigate constraints affecting a donor funded development project developing transition of pond based subsistence fish farming to small scale commercial within five districts of Southern Malawi, based on careful selection criteria involving small numbers of entrepreneurial fish farmers. The in country project manager was recruited from the private sector with many years experience working in and managing commercial tilapia farms and hatcheries in Zimbabwe and Uganda

Baseline survey conducted in May 2013 involving 312 fish farmers within the districts of Blantyre, Zomba, Thyolo, Chikwawa, Chiradzulu, Mulanje and Phalombe gave a clear understanding of the situation prior to project implementation. There was minimal inclusion of government ministries and department staff within the project to ensure there was a strong commercial and small business development drive to the project. Umodzi Consulting was one of the key partners. It is a specialist small to medium scale agricultural business development consultancy based in Blantyre. A total of sixty fish farmers were carefully selected over three years based on the most important factors that derives successful fish farming. Inputs loans were offered to the fish farmers to allow them to have 6 months formulated feed which a significant majority of the fish farmers were able to pay back from their harvest incomes. This feed was approximately 70 -75% of their 6 month production cycle running costs By the end of July 2016 (Year 3) the project was working with 44 fish farmers out of the 60 fish farmers initially selected. Inputs loan repayment for their formulated feed was 90% clearly indicating these production systems if managed properly could be profitable. Regular on site coaching and mentorship from suitably experienced project staff on business management and technical aspects of pond culture enabled the AEM project fish farmers to register such success.

The project also had a clearly defined exit strategy grouping its fish farmers into 5 geographically located clusters (not associations) in five districts that enabled them to share fish farming knowledge, marketing information, bargaining power and their own resources to procure seed and fish feed themselves to ensure future economic viability and in some cases further expansion. As a different, cost effective and value for money project model, a different approach to the past norms which on initial findings has shown pond cultivation of indigenous native tilapias in southern Malawi can be a financially viable income generating activity with the right building blocks and project infrastructure put into place replication in other locations is highly recommended.

## **FISH FARM WATER TREATMENT: NEW MULTI-APPROACH FOR APPLICATION OF SODIUM FERRATE**

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Worldwide is well known as the aquaculture sector is very promising: an important food security tool, a great business opportunity and a possibility of work for a number of technician and workers. This is not only the writer opinion but is also for other author as Peter Drucker( Management Guru) that state “Aquaculture, not the Internet, represents the most promising investment opportunity of the 21st Century.” and others.

The fast increase of the world aquaculture activities has greatly developed. Advanced technologies permit the increased densities of the farmed animals but increased the wastewater production adding some element of difficult elimination as residues, disinfectant and other molecules (antibiotics, additive, ...).

A large array of technologies is available to treat the aquaculture inlet and effluent water, to permit the appropriate, economic and to respect to the legislation framework of the aquatic farming. In fact the water used for aquaculture become a limited factor for his scarcity, cost and in competition with other human activities, the same for the land available. The above obliges a good use of the available water, appropriate water and wastewater treatment and possibly recycling with RAS (Recirculating Aquaculture System) technologies.

Ferrate (VI) is proposed as an interesting solution for a more performing aquatic animal farming. The tests already done with sodium ferrate (Fe VI) showed optimal use of this chemical for the aquaculture for wastewater and organic and inorganic disinfection. Until now very few authors provided information about the use of sodium ferrate in aquaculture.

Several technologies are available but the sodium ferrate shows the most promising technological characteristics, thus a new patented machinery model is presented in this paper.

The increasing concentration of farmed aquatic animals needs appropriates, clean and green technologies for water and waste water treatment to coagulate, chemical oxidation and disinfection. Ferrate (VI) is a green chemical for the above mentioned porpoises.

This paper present some patented equipment for ferrate (VI) production and utilization in coagulation, chemical oxidation and disinfection of water and wastewater treatment.

In good conditions (better acidic 6-8 PH, but still good in others), the oxidation and reduction capacity of ferrate (VI) is superior to all currently utilized oxidizers and disinfectants in water and wastewater treatment

### **INTRODUCTION**

Worldwide is well known as the aquaculture sector is very promising: an important food security tool, a great business opportunity and a possibility of work for a number of technician and workers. This is not only the writer opinion but is also for other author as Peter Drucker( Management Guru) that state “Aquaculture, not the Internet, represents the most promising investment opportunity of the 21st Century.” and others.

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Ferrate (VI) is proposed as an interesting solution for a more performing aquatic animal farming. The tests already done with sodium ferrate (Fe VI) showed optimal use of this chemical for the aquaculture for wastewater and organic and inorganic disinfection<sup>1</sup>. Until now very few authors provided information about the use of sodium ferrate in aquaculture.

Several technologies are available but the sodium ferrate shows the most promising technological characteristics, thus a new patented machinery model is presented in this paper.

From the figure one it is clearly showed the following world trend: half of the fish stock is overexploited, the wild fish production is stable and difficultly will increase, shrimp farming that represent almost the 50% of the world production is concentrated in Asia. Fish farming will continuously increase during the next year, the feed conversion ratio of aquatic animals is more favourable of the other farmed animals. All the above is a clear statement of the future increase of the world aquaculture activities, but space and water are limited and intensive aquaculture system is in more need.

## CONTEXT

The modern aquaculture systems could be divided in two main type open and closed systems. In the first one the water used is discharged in the environment, after being used in the farm; in the closed system a part (rarely all the water) of the water is recirculated after specific treatment. In the first case the solids and dissolved nutrients is discharged in the environment.

The production of aquatic animals usually produce wastes composed of:

- Nitrogen (N)
- Phosphorous (P)
- Carbon C
- Ortho-phosphate (PO<sub>4</sub>)
- Solubles waste – carbon dioxide (CO<sub>2</sub>)
- Ammonia (TAN)
- Residues and trace elements (antibiotics, heavy metal, disinfectant, ...)

There are some treatment system to avoid nuisance and environmental impact from the wastes:

- Bacterial that degrade ammonia into other organic N, urea and Nitrate (NO<sub>3</sub>)
- Plant that use the wastes to grow and transform into biomass

Micro and macro algae that use the sun and the waste to transform in biomass

All the above treatment system has his own advantage and disadvantage and ferrate can be used together to optimise the water and wastewater results.

The ferrate can be used as an easy, reliable and economic product to be used in aquaculture and fish processing for an efficient and efficacy water use and reuse. The ferrate use is innovative for farm effluent (but could be used also in the inlet water if needed) treatment, water reuse and by products elimination in the agro industry.

The increasing concentration of farmed aquatic animals needs appropriates, clean and green technologies for water and waste water treatment to coagulate, chemical oxidation and disinfection. Ferrate (VI) is a green chemical for the above mentioned porpoises.

This paper present some patented equipment for ferrate (VI) production and utilization in coagulation, chemical oxidation and disinfection of water and wastewater treatment.

In good conditions (better acidic 6-8 PH, but still good in others), the oxidation and reduction capacity of ferrate (VI) is superior to all currently utilized oxidizers and disinfectants in water and wastewater treatment<sup>2</sup>.

(Continued on next page)

Comparison of different products of current disinfection and oxidant capability treatment of water and wastewater

New ferrate patented technologies now permit the coagulation, chemical oxidation and disinfection of water and wastewater simultaneously in a reactor. The above permit the reduction of the size of water and wastewater equipment's increasing the efficiencies of their effluent treatments.

Moreover the ferrate equipment could treat the inlet or recirculating aquaculture farm water solving a number of problems and increasing the farm productivity.

Until now few large scale patented equipment are under test in the aquaculture sector, the ferrate (VI) efficacy is already tested and demonstrated for disinfection against main aquaculture virus, spore, bacteria and parasites, ..... (REF ...).

Moreover ferrate (VI) is very effective in degradation of heavy metal, PCB, pesticide, antibiotics and other residues.

It is well known as diseases (bacterial, virus and parasites) provide problems particularly to the aquaculture industry (finfish, crustacean and others) with food security and important economics losses providing. Climatic change could exacerbate the above losses<sup>3</sup>. The new ferrate technologies could be considered as an important mitigation and be adapted as an important strategy that could be considered globally to contrast the farming disease vulnerability and consequent risks.

Ferrate (VI) water and wastewater difficulties were solved with the new patented equipment as is already tested in laboratory and new large scale field tests are ongoing showing advantages over other methods. The new equipment permit the ferrate (VI) production; this overcome the ferrate (VI) product instability now adapted to the water environment and an appropriate production yield appropriate to the large water volume to be treated in the aquaculture farms. The ferrate (VI) production yield and stability of the new patented equipment permit from now industrial use in the aquaculture farms.

Additionally it is forecasted other studies on live fish and crustacean that could provide additional benefits contrasting the most common pathologies to the aquaculture industry.

## EU LEGISLATION CONCERNING FERRATES

It is not a biocide and can be used according with the author opinion.

## GREEN CHEMISTRY

The Green Chemistry high light chemical processes by primarily considering safety, pollution prevention, waste minimization and energy optimization. One of the main goal of green chemistry is to provide a solid solution to the need of the industrial process without harming the environment.

Green chemistry considers not only the safety of a chemical procedure, but also the proper selection of solvents, starting materials, and technologies used to generate and control reactive intermediates. In addition green chemistry considers the need for minimizing toxic waste and the respective disposal cost highlights how crucial it is to consider the recovery and reuse of the materials needed for a synthetic process<sup>4</sup>.

Lower CO<sub>2</sub> consumption is another aspect to be considered in the chemical reaction and in the production of the inputs and equipment for the proposed process. Ferrate (VI) is considered a green chemistry product<sup>5</sup>

Throughout the last ten years, several experimental works about the Sodium and Potassium Ferrate laboratory and pilot production process and their application have been published. This family of products showed a very high oxidant capacity, for the state of oxidation of the iron (VI) which sparked a remarkable interest in its applications both for disinfection and for especially complicated water treatments (landfill leachate, arsenic removal, ammonia, surfactant removal, phosphorus removal, etc. )

*(Continued on next page)*

<sup>3</sup> More rapid and severe disease outbreaks for aquaculture at the tropics: implications for food security Tommy L. F. Leung<sup>1</sup> \* and Amanda E. Bates, Journal of Applied Ecology 2013, 50, 215–222

<sup>4</sup> Green Chemistry, Beilstein Journal of Organic Chemistry **2016**, 12, 2763–2765.doi:10.3762/bjoc.12.273 – Luigi Vaccaro,

<sup>5</sup> <http://dx.doi.org/10.1016/j.jece.2017.03.025>, Op. cited

Furthermore, a lot of research has been conducted on the by-products of this reaction and especially on the formation of Iron Hydroxides, and their absolute absence of residual toxicity.

This work, reports experimental evidences of an innovative process that allows the use of Sodium Ferrate in quantities appropriate for industrial use and introduce the main known and consolidated applications, with a specific focus on Fisheries process requirements.

## FERRATES AND REACH

Sodium Ferrate is pre – registered with EC list number 917-620-0 in REACH list at EU level

## FERRATES CHEMICAL NATURE AND GENERAL PROPERTIES

### OXIDISING CAPACITY

Fe (VI) is a strong oxidant agent. The redox potential of ferrate is higher than ozone under acidic conditions and is the highest of all the oxidant disinfectants used for water and wastewater treatment.

Several halogen and oxygen-based oxidants are widely used, but each one of them has limitations with respect to the production of by products. During oxidation, ferrate also generates a base (OH<sup>-</sup>) in aqueous solution, removing metal ions present as a result of hydroxide precipitation.

Studies in the use of ferrate as an oxidant have shown that it can remove organic pollutants and effectively treat nitrogen and sulfur-containing contaminants in water and wastewater effluents by oxidizing them into harmless products. The extent of organic compounds oxidation strongly depends on the ferrate dose.

The ferrate (VI) is maybe the most powerful oxidative component, which can be used in oxidative applications, by showing an oxidation potential of 2.2 volts in acid environment.

Fe(VI) shows excellent disinfectant properties and can inactivate a wide variety of microorganisms at low Fe(VI) doses.

Fe(VI) also possesses efficient coagulation properties and enhanced coagulation.

The final product of Fe(VI) reduction is Fe(III), a non-toxic compound.

Moreover, treatments by Fe(VI) do not give any mutagenic/carcinogenic by-products, which make ferrates environmentally friendly ions.

During oxidation of organic matter and microorganisms in water, ferrate (VI) will be reduced to ferric (III), generating a coagulant that has proven to reduce turbidity of water and decrease the concentration of natural organic matter. ***One of the benefits of the use of ferrate for water and wastewater treatment is that lower doses of ferrate are needed when compared with other coagulant agents and thus the sludge generation is reduced.*** Another advantage of ferrate is that it can destabilize colloidal particles within 1 minute.

In wastewater treatment, ferrate (VI) can remove 50% more colour and 30% more chemical oxygen demand (COD) in comparison with commonly used coagulants, ferric sulphate and aluminium sulphate at the same or smaller doses.

### DISINFECTING CAPACITY

Since the discovery of chlorinated by-products (CBP) and their negative health effects, great efforts have been made to minimize the CBP formation after disinfection with chlorine or other halogens.

*(Continued on next page)*



Ferrate in addition to its oxidant and coagulant powers, acts as disinfectant that does not react with organic matter to form carcinogenic tri-halomethanes (THM). Since the first observation of the abilities of the ferrate to kill and inactivate bacteria and viruses, many studies have also proven that it can retard the growth of biofilms, and serves as an anti-fouling agent. Ferrate strong oxidizing bacteria can destroy the cell wall, cell membrane and cell structure of the enzyme, inhibition of protein and nucleic acid synthesis, impeding bacteria growth and reproduction of the body, play a role in killing bacteria. ferrate concentration is 10 ~ 40 mg / L, the contact time of 5 min or multiplication of bacteria such as *E. coli*, *Staphylococcus aureus* more strong role in the killing, the killing rate was 100%; of fungi also have some role in the killing, the killing rate was 99.50% or more (Wang Kaijuan)

Tanchang Sen et al [18] describe the disinfection efficiency of potassium to iron in water samples medium to a concentration of 5.6 ~ 6.0 mg / L at values 99.95% to 99.99%.

Researchers have shown that for a low dose of ferrate (10 mg/L as Fe), approximately two logs of inactivation of total bacteria were observed<sup>6</sup>.

Iron compounds in the oxidation state (VI) have the advantage of being powerful antioxidants and bactericides, which explains their particular interest in water treatment.

In many technical paper are reported the biocidal capacities against *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, *Bacillus sp.*, *Pseudomonas sp.*, *Enterococcus faecalis* by ferrates.

## SPECIFIC APPLICATION IN AQUACULTURE

It is well know that fish diseases and water quality of farmed fish is an important factor in yield, pathology prevention and quality.

In aquaculture production process, it has wide applied the use of chemical disinfectants for disinfection of water in order to achieve the purpose of prevention and control of aquatic animal diseases. However, chemical disinfection to the water splashing agent will break the water microbial community structure, influence the balance between organisms, directly or indirectly, adversely affect aquatic animals; some chemical disinfectants in the disinfection process of decomposition products have mutagenic, carcinogenic effects, can aquatic animal and human health, cause serious harm.

The introduction of new efficiency and low toxicity residue to control disease or improve pharmaceutical farming water ecological environment is everywhere in development.

Especially, it is available a documentation related to a project carried out in UK and Scottish aquaculture sector regarding the impact of control methods on the main diffuses fish diseases<sup>7</sup>.

The purpose of this project was:

- to identify the key diseases that affect freshwater aquaculture operations in Scotland, particularly the trout sector, and determine their relative impact;
- to identify the main methods used to control these diseases;
- to identify the potential consequences if any of the main control methods were to be withdrawn; and finally,
- to identify any new potential treatments that could be used instead, if any of the main treatments were to be withdrawn<sup>7</sup>.

Producers, vets and health professionals surveyed confirmed that production was constrained by a limited group of common diseases that affected rainbow trout producers in England and Scotland. These included rainbow trout fry syndrome (RTFS) caused by the bacterium *Flavobacterium psychrophilum*, white spot disease caused by the endoparasite *Ichthyophonus multifiliis*, enteric red mouth disease (ERM) caused by the bacterium *Yersinia ruckeri*, proliferative kidney disease caused by the myxozoan parasite *Tetracapsuoidesa bryosalmonae*, red mark syndrome (RMS) and bacterial gill disease (BGD).

Possible alternatives to the use of dangerous formalin products purchased for biocidal applications were reviewed in the event of their withdrawal from sale.

(Continued on next page)

<sup>6</sup> An Overview on Production and Application of Ferrate (VI) for Chemical Oxidation, Coagulation and Disinfection of Water and Wastewater Amirreza Talaiekhazani, "Mohammad Reza Talaei, "Shahabaldin Rezania"<sup>d</sup>

<sup>7</sup> SARF 100 - Review of freshwater treatments used in the Scottish freshwater rainbow trout aquaculture industry, David Verner-Jeffreys CEFAS - 2015

Review of the literature suggested that **FERRATES** may all have some promise as alternative treatments.

Potassium and Sodium Ferrate ( $\text{Me}_2\text{FeO}_4$ ) has been identified as a potentially effective white spot treatment in a recent review (Picón-Camacho *et al.*, 2012)<sup>8</sup>. It is a strong oxidising agent which has non-toxic break down products (Fe III and oxygen). Recent work has shown that 4.8 mg/l potassium ferrate (VI) for 2 hours was very effective *in vitro* in killing theronts and, when applied continuously over 3 days *in vivo*, caused an 80% measured reduction in the numbers of trophonts on the test fish. A dose of 19.2 mg/l for 3 days resulted in complete clearance of infection in treated gold fish<sup>9</sup> and <sup>10</sup>. It has been identified as a chemical for use in waste water treatment applications, due to its reported high stability, strong oxidising power and limited environmental impact<sup>11</sup>. However, to this author's knowledge, no potassium ferrate product is marketed for use as a biocide in the EU now and it is not listed as EU Regulation No 37/2010.

This depends on the fact that Ferrates are not BIOCIDES but they have a strong biocidal efficiency without generation of toxic by-products.

That will avoid to the user to carry out a complex analysis plan of residual Biocide determination into the marketed product

The pollutants generated by aquaculture are mainly nitrogen, phosphorus, and causing serious environmental problems. Therefore, treating these pollutants is very much essential for successful aquaculture. Therefore, it is important in aquaculture using water reuse systems due to the toxicity of ammonia and nitrite and the chance of hypertrophication of the environment by nitrate. Moreover, the superior performance of ferrate (VI) as an oxidant/ disinfectant and coagulant in water and wastewater treatment has been reported. Therefore, ferrate based treatment for aquaculture wastewater treatment will be a milestone in terms of economics, technical feasibility and social acceptability.

The ferrate treatment system has certain advantages such as precipitation of phosphorus, removes heavy metals, kills spores, bacteria, viruses and protozoa, produces no aquatic toxicity, deactivates residual drugs & pesticides, reduces organic load in the water body which reduces biochemical oxygen demand, and removes color & odors.

The final product of  $\text{Fe}(\text{OH})_3$  or  $\text{Fe}(\text{OH})_6$  is nontoxic and environmentally acceptable and can be easily filtered or settled without creating any toxicity to the surroundings. Moreover, there are certain challenges associated with the use of ferrate treatment strategy.

The challenges associated with the use of the proposed technology includes, Fe (VI) solutions are generally unstable; It decomposed by reduction to Fe(III) rapidly at room temperature. The instability may be retarded at low temperature. Therefore, without refrigeration the Fe (VI) solutions cannot be practically stored for long time. This problem can be minimized to generate ferrate in situ and apply the generated ferrate (VI) directly for wastewater and other treatment.

## PHOSPHORUS REMOVAL

Current phosphorus removal practice employs biological, chemical, or combined biological and chemical processes. Biological phosphorus removal relies on the function of a specific group of polyphosphate-accumulating microorganisms that are capable of taking up excessive phosphorus as intracellular storage, and the phosphorus is removed from the liquid by sludge wasting.

Chemical phosphorus removal is achieved by the addition of salts of multivalent metal ions (e.g.  $\text{FeCl}_3$ ,  $\text{Fe}_2(\text{SO}_4)_3$ ,  $\text{Al}_2(\text{SO}_4)_3$ , or  $\text{Ca}(\text{OH})_2$ ) to form precipitates of sparingly soluble metal phosphate complexes. Both of these processes target at eliminating only soluble orthophosphates or those forms in the influent that can convert into ortho-P during the treatment process, by transforming it into solids phase followed by subsequent solid and liquid separations. Since most permit limits are based on total phosphorus (TP), the effluent P level is affected by both the effectiveness of chemical and/or biological P treatment processes as well as the final solid and liquid separation efficiencies.

Many technical studies are reporting that Ferrates(VI) was applied to treat secondary wastewater and its performance as both a disinfectant and a coagulant was investigated.

(Continued on next page)

<sup>8</sup> An Assessment of the Use of Drug and Non-Drug Interventions in the Treatment of Ichthyophthirius Multifiliis Fouquet, 1876, a Protozoan Parasite of Freshwater Fish, SM Picón-Camacho *et al.* Parasitology 139 (2), 149-190. 2011 Nov 14

<sup>9</sup> Prevention of Ichthyophthirius multifiliis infestation in goldfish (*C. auratus*) by potassium ferrate (VI) treatment, Fei Ling *et al.* Laboratory of Healthy Aquaculture, Institute of hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China, Veterinary Parasitology – 168(2010) 212-216

<sup>10</sup> Treatment of Ichthyophthiriasis in Rainbow Trout and Common Carp with Common and Alternative Therapeutics  
Article in Journal of Aquatic Animal Health 19(3):186-94 · October 2007, Franz Lahnsteiner and Thomas Weismann

<sup>11</sup> SARF 100 Review of Freshwater Treatments

In order to compare oxidation potential of the ferrate(VI) with that of chlorine, the different condition of doses and contact times were applied to the experiment for the same coliform. The disinfection rate of ferrate(IV) was faster than that of chlorine at the same concentration. The effect of ferrate(VI) supplementation on coagulation of phosphorus was examined and compared with other common coagulants.

Ferrate(VI) reduced more than 80% of total phosphorous in the range of doses between 5. and 25 mg-Fe/L. The removal efficiencies of the ferrate(VI) appeared to be similar to those of alum but higher than other iron coagulants.

Example of continuous Ferrate generators are available for sewage treatment, from pilot to full-scale trials at Hailsham North Wastewater Treatment Plant of Southern Water Ltd of UK. The work presented in the paper has significant impact on the use of ferrate (VI) in water and wastewater treatment practice; the online production and application of ferrate (VI) resolves problems of the instability and needs no transportation.

For achieving the same phosphorus removal target from the crude sewage, the ferrate dose required was in a very lower range, 0.01 - 0.2 mg Fe<sub>6</sub>/L in comparison with high doses of ferric sulphate; these will reduce the chemical demand and sludge production and therefore result in a low operating cost and generate substantial cost saving in treating sewage.

As final consideration, it is useful to remark that in Switzerland, Ferrates represent a novel technology for enhanced municipal waste water treatment based on the dual functions of Fe(VI) to oxidize micro pollutants and remove phosphate by formation of ferric phosphates. Second-order rate constants (k) for the reactions of selected pharmaceuticals, endocrine disruptors, and organic model compounds with Fe (VI) were in the range of 1 (trimethylamine) to 9000 M<sup>-1</sup> s<sup>-1</sup> (aniline) in the pH-range 7–8. The selected compounds contained electron-rich moieties (ERM) such as phenols, anilines, amines, and olefins. Oxidation experiments in wastewater spiked with micro pollutants at concentrations in the low µM range at pH 7 and 8 showed that Fe(VI) doses higher than 5 mg Fe L<sup>-1</sup> are capable of eliminating various ERM-containing micro pollutants by more than 85%. In comparison to ozone, Fe (VI) was as effective or slightly less effective in terms of micro pollutants oxidation, with Fe(VI) having the benefit of phosphate removal. To lower phosphate from 3.5 to 0.8 mg PO<sub>4</sub>-P L<sup>-1</sup> (regulatory limit for wastewater discharge in Switzerland), a Fe(VI) dose of 7.5 mg Fe L<sup>-1</sup> was needed. Overall, this study demonstrates Fe (VI) as a promising tool for an enhanced wastewater treatment to remove micro pollutants as well as to control phosphate in a single treatment step.

Obviously, for Aquaculture sector, that represent ad added value and can represent a winning factor in the product marketing.

## BASIC PRINCIPLES IN ON SITE GENERATOR PRODUCTION METHOD

The two most common processes for ferrate production are:

Wet Method through a Reaction between iron salts and hypochlorite. This method has some undesirable effects:

The use of hypochlorite does not produce a “chlorine free” product.

The reaction is not quantitative, so the resulting flow is a mixture of reagents and reaction products.

Electrochemical method in electro-cells, either not separated or separated from membranes. The most difficult aspect of this method in the ferrate production is the passivation of the anode, caused by the formation of a ferric oxide film on the iron anode.

## CONCLUSION

The new INTECNA's process described in this document protected by PATENT **2013 A 001804**, allows the production of sodium ferrate, using an electrolytic cell which has one sector (anolyte) containing an anode, and a second sector (catholyte) containing a cathode.

Sectors are separated from a conductive membrane.

For the application in AQUACULTURE, Intecna proposes a plant for the Sodium Ferrate production to be installed on site.

*(Continued on next page)*

Sodium ferrate will be generated in the plant by electrochemical oxidation of Iron specific electrodes in a proprietary electro-cell design accordingly to the Patent mentioned in this technical paper.

Depending on the required daily production, new patented design Ferrate machinery to proceed in form of Batches by applying a small safety over dimensioning to face possible maintenance or shut down stops.

The developed process allowed optimizing the cell manufacturing aspects and operating processes which results in the designing of a generator for a very pure ferrate production in an industrial setting.

The use of the ferrate contextually to its production also allows the user to keep production costs very low thanks to the small size of the generator. In addition to these advantages, this process makes it possible to remove the inconveniences of storage of a fast reactive product.

In conclusion, the generation of Sodium Ferrate involves only the use of especially designed iron electrodes, the manufacturing and the use of a special membrane and the generator feeding with high purity sodium hydroxide. Thanks to this technology, an excellent compromise between costs and performances and complete no toxic effect on fish is achieved.

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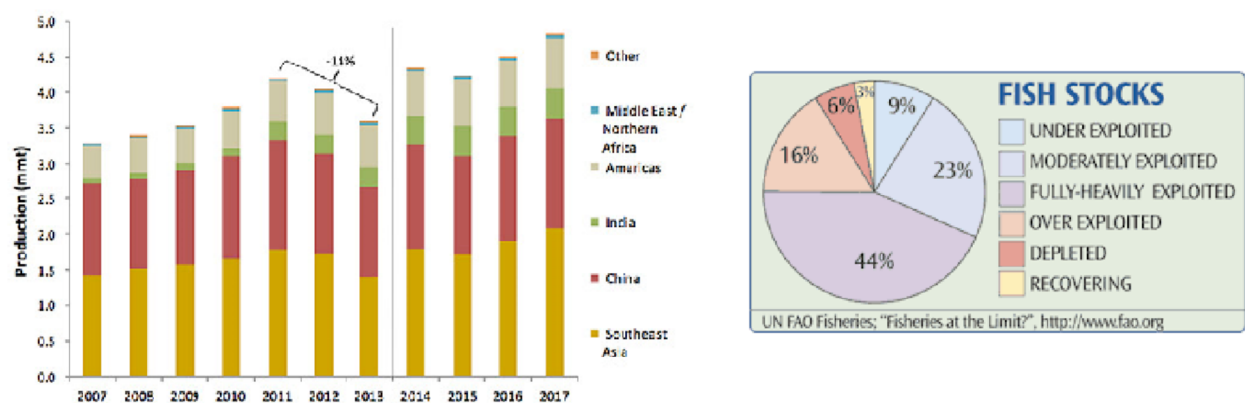


Figure 1: Shrimp farming production by region. Sources: FAO (2007-2011); FAO & GOAL 2014 (2012-2013); GOAL 2015 (2014-2017).

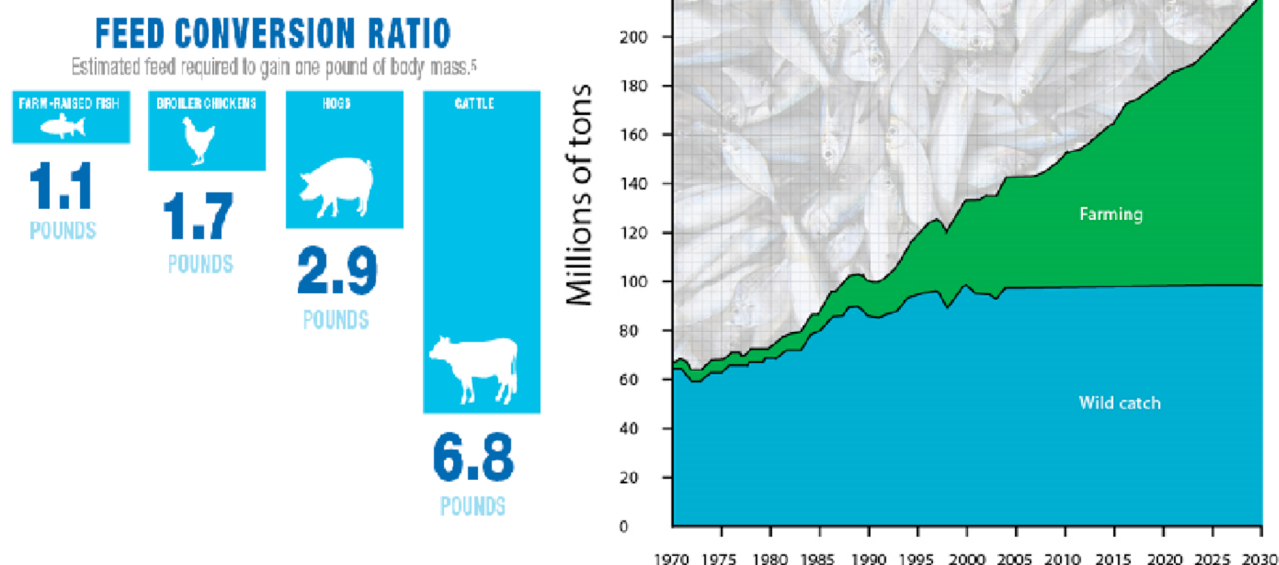
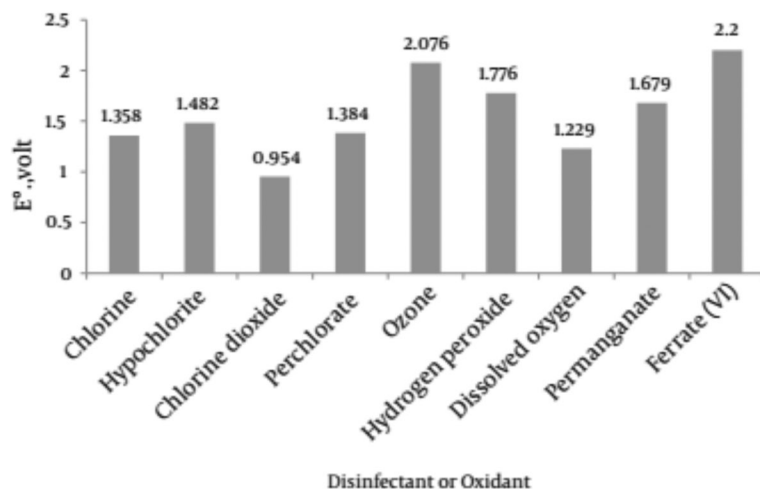


Figure 1: Fishery versus aquaculture production  
Source : <http://www.iraishrimp.com/overview.html>

Comparison of different products of current disinfection and oxidant capability treatment of water and wastewater



Source: A. Talaiekhazani et Al.<sup>2</sup>

(Continued on next page)



**Table 1: The ferrate machinery considerations for the green chemistry are the following**

| <b>Principle of Green chemistry</b>                    | <b>Ferrates respects of principles</b>              |
|--|---|
| 1. Pollution Prevention                                | Yes   |
| 2. Atom Economy  | Yes   |
| 3. Less Hazardous Chemical Synthesis                   | Yes   |
| 4. Designing Safer Chemicals                           | Yes   |
| 5. Safer Solvents and Auxiliaries                      | Yes   |
| 6. Design for Energy Efficiency                        | Yes (possibility to use alternative energy sources) |
| 7. Use of Renewable Feedstocks                         | Yes   |
| 8. Reduce Derivatives                                  | Yes   |
| 9. Catalysis   | Yes   |
| 10. Design for Degradation                             | Yes   |
| 11. Real-time analysis for Pollution Prevention        | Yes   |
| 12. Inherently Safer Chemistry for Accident Prevention | Yes   |

Source: Author consideration and L. Vaccaro article<sup>4</sup>

The table below shows the Potential REDOX of the most common oxidizers.

| <i>Oxidant</i>           | <i>E°, V (Basic)</i> | <i>E°, V (Acidic)</i> |
|--------------------------|----------------------|-----------------------|
| <b>Chlorine</b>          | 1.358                | -                     |
| <b>Hypochlorite</b>      | 1.482                | 0.841                 |
| <b>Chlorine Dioxide</b>  | 0.954                | -                     |
| <b>Perchlorate</b>       | 1.389                | -                     |
| <b>Ozone</b>             | 2.076                | 1.240                 |
| <b>Hydrogen peroxide</b> | 1.776                | 0.880                 |
| <b>Dissolved Oxygen</b>  | 1.229                | 0.400                 |
| <b>Permanganate</b>      | 1.679                | 0.588                 |
| <b>Ferrate(VI)</b>       | 0.70                 | 2.20                  |

## THE EFFECT OF KELP *Ecklonia maxima* INCLUSION IN FORMULATED FEED ON GROWTH, FEED UTILISATION AND THE GUT MICROBIOTA OF SOUTH AFRICAN ABALONE *Haliotis midae*

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Kelp *Ecklonia maxima* is included in formulated abalone feeds in South Africa, but its effect on abalone growth, feed utilisation efficiency and gut-bacterial communities has not previously been investigated. An eight-month on-farm growth trial with sub-adult *Haliotis midae* (~43 mm shell length) fed graded levels of kelp in formulated feeds was conducted. Kelp inclusion (0.44 – 3.54 % of pellet dry mass) promoted faster growth (65.7 – 74.5 % total mass gain), with better feed and protein conversions (FCR: 1.4 – 1.8; PER 2.3 – 2.7), compared to abalone fed the non-supplemented feed (52.3 % total mass gain; FCR: 2.1; PER 1.9;  $p < 0.001$ ).

The gut-bacterial communities of abalone fed kelp-supplemented feed (0.88 % of pellet dry mass) were subsequently compared with that of abalone fed a non-supplemented control diet. Abalone gut-bacterial DNA was sequenced using 16S rRNA pyrosequencing and sequences were clustered into operational taxonomic units (OTUs) at a 97 % similarity level. A supplementary 16S rRNA denaturing gradient gel electrophoresis (DGGE) analysis was conducted. The dominant OTUs differed in terms of their relative abundances, with an autochthonous Mollicutes strain being significantly higher ( $p = 0.03$ ) in the guts of abalone fed kelp-supplemented feed. The DGGE band patterns displayed a higher within-group variability of dominant bacterial strains for abalone fed the control diet, suggesting that dietary inclusion of kelp, which is rich in fermentable polysaccharides, promotes a balanced gut-bacterial community. This may contribute to the better feed utilisation and growth in abalone fed kelp-supplemented feeds.

## AQUATIC ANIMAL HEALTH STATUS IN BOTSWANA

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Historically, aquatic animal diseases have not been an issue of concern in Botswana until the first outbreaks of Epizootic Ulcerative Syndrome (EUS) in the Chobe/Zambezi riverine system in 2006. The outbreaks resulted in the first diagnosis of EUS in Africa in Chobe river in Botswana. The origin of the disease and how it ended up in the Chobe/Zambezi basin still remains a mystery. The disease was first diagnosed in Asia and Australia in the early 1970's and it then spread to other countries from there. Most of the natural fish mortalities were related to water quality, especially fish kills associated with dissolved oxygen depletion. Introduction of exotic species can easily be done through aquaculture, either through fish farming, ornamental trade or through deliberate initiatives or escapees. As a way of improving food security and nutritional standard of marginalized communities, the government is currently embarking upon a poverty eradication program whose main objective is to promoting fish farming by citizens. Through fish farming, fish diseases that are not within the country can be introduced with fish that are brought in for farming if adequate control measures are not in place.

Ornamental fish intended for aquaria purposes poses great threat to transmission of diseases into the country. There is a lot of ornamental fish that is being imported into the country there is no monitoring mechanisms in place nor any regulatory measures that control fish movement into and within the country. Some exotic fish species are being introduced by unscrupulous people by different people for various purposes such as promotion of angling activities and enhancement of water bodies to increase fish productivity. Some of the examples include the introduction of largemouth bass (*Micropterus salmoides*) in Letsibogo dam and Nywaane dam for sport fishing. There are currently reports of the introduction of the Redclaw crayfish (*Cherax quadricarinatus*) in the Zambezi river system. The main river systems in Botswana, viz; Okavango (shared with Angola and Botswana), Chobe/Zambezi basin (shared with Namibia, Zambia and Zimbabwe), Limpopo river (shared with Zimbabwe and South Africa), Nata river (shared with Zimbabwe) are trans-boundary in nature. Therefore, any disease brought into any of these countries may end up in Botswana; hence protocols or strategies on aquatic animal health need to be established amongst riparian states on these shared watercourses.

### What needs to be done?

- Development of a National Aquatic Animal Health Strategy
- Development of a National Aquatic Animal Health Risk Analysis Framework
- Conduct Risk Analysis Assessment for any aquatic fish species that need introduction into the country

## FISH VALUE CHAIN ISSUES IN BOTSWANA

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The presentation takes a look at different components of the fish value chain, as well as some general economic considerations for an industry in its infancy.

During a training exercise on value chain and marketing, held in northern Botswana for the Office of the President end of last year, local fishermen and prospective fish farmers were encouraged to think “chain” and see a variety of economic opportunities, not only in the production of fish itself.

Botswana is a land locked country with no natural lakes and really only 2 rivers with perennial water (Okavango and Chobe). Earlier, locally produced fish was dried and salted or sometimes smoked. Many parts of the country only saw fish in cans. Today, with improved infrastructure all major villages have supermarkets/shops with deep freezers and fish in frozen form is available in many places countrywide.

The trade of fresh fish (on ice) in Botswana is almost nonexistent. This is mainly due to the fact that fish is caught/produced far away from markets. Most consumers consider fresh fish to mean not yet cooked, whether it has been frozen or not.

At present there is only one commercial bream farm in Botswana, located in the very north of the country. Part of their product is sold straight to the tourism/hospitality industry and part is sold scaled, gutted, frozen and packed in branded plastic bags (5 fish per kg). The product is sold in supermarkets at a price of about USD 6.55/kg (Botswana Pula 68.00).

Due to an increased interest shown in the aquaculture sector, the Local Enterprise Authority (LEA) undertook a Fisheries Value Chain Analysis Study in Botswana in 2015. The recommendations from that include the following;

An incubation centre for fish farmers, establishment of mobile fish collection points with cold storage facilities and a fish processing plant.

It also stated that government needs to create the enabling environment by implementing the Aquaculture Strategy for Botswana.

Apart from such physical developments the fish producers will need education and training in the value chain concept; processing, branding, pricing, marketing and distribution.

## EFFORTS TO DEVELOP THE AQUACULTURE SECTOR IN BOTSWANA

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The presentation takes a look at the major programs and projects that have been undertaken in support of aquaculture development in Botswana since 1990.

Aquaculture for Local Community Development Program was a FAO program that covered all the SADC countries. The aim of the project was to improve the living standards of the rural poor through the practice of aquaculture. In Botswana the project was implemented through the Fisheries section, Ministry of Agriculture from 1990 to 1993. The main focus was on the fisheries resource in 7 smaller dams in the south/southeastern part of the country. Through a full year test-fishing exercise the productivity was estimated. The program listed a series of management options for the different dams, with consideration to the size of dams, geographic location and water utilization.

In 2004 the Botswana College of Agriculture began a course Introduction to Aquaculture for higher national diploma students. Many of the students were in training to become agricultural teachers at secondary school level. The Fisheries Unit started a process to lobby for the construction of a hatchery already in mid 90s. The intention was to produce bream and catfish fingerlings for stocking of dams as well as for supply to future fish farms. This effort came to fruition in 2006 when the hatchery started its operation. Due to staff constraints, management could not keep the hatchery operational and it has not produced much fish during the last 5 years.

Fisheries and aquaculture was addressed at national level in 2008 during a FAO exercise to produce a National Report for Food Security. The report had recommendations for some aquaculture projects.

In 2008 a consultancy was commissioned to produce the Guidelines for Aquaculture Development for Okavango. As Okavango is a sensitive ecological area, it was regarded as important to have guidelines to advice on any future aquaculture developments.

Aquaculture was again addressed in 2011 when a draft National Fisheries Policy of Botswana was prepared. This is still in a draft form but it has been used as a guiding document for planning purposes.

Due to an increased interest shown in aquaculture as a concept, the Local Enterprise Authority undertook a Fisheries Value Chain Analysis Study in Botswana in 2015.

## DIFFERENCE IN TRANSCRIPTOMIC RESPONSE OF *Salmo salar* SKIN ASSOCIATED TO CHALIMUS STAGE OF THE CHILEAN SEA LOUSE *Caligus rogercresseyi* USING RNA-SEQUENCING

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The sea louse *Caligus rogercresseyi* is the most important copepod ectoparasite of Chilean salmon industry. The impact of sea louse is related to loss of product quality, mainly due to the parasite's activities of both settling and feeding on the skin, which can increase susceptibility to other pathogens. In order to better understand the biological mechanisms involved in the response of the host to sea lice, the main objective of this study was to characterize the transcriptome profile of *Salmo salar* skin in response to *C. rogercresseyi* experimental infestation, at chalimus stage of development using RNA-Sequencing

A challenge trial was conducted on the pedigree population of 2355 *S. salar* from the genetic program of AquaGen Chile S.A. (Puerto Varas, Chile) which were exposed to *C. rogercresseyi* in its infestive stage (copepodid). A total of 84000 copepodids were used, which were obtained from a local strain of the parasite in Puerto Montt, Chile. The stage of development was monitored and the sea lice was allowed to develop until the chalimus III – IV stage. Chalimus counts were conducted on all fish, and individuals with extreme phenotypes were classified as having low (L) or high (H) lice count. Skin samples were taken from the area below the dorsal fin, at the height of the lateral line. The samples were RNA-sequenced on a MiSeq platform (Illumina®, USA). Raw RNA-seq reads were quality controlled and then mapped to the reference genome of *S. salar* (GCF\_000233375.1\_ICASG\_v2) using TopHat (v2.1.1). Differential gene expression (DE) analysis was performed with the R package EdgeR. Genes were considered to be differentially expressed with a false discovery rate (FDR)  $p\text{-value} \leq 0.05$ . The enrichment analysis on Gene Ontology (GO) terms were performed using the DAVID with a corrected  $p\text{-value} \leq 0.05$ . The most representative subset of GO terms was determined with the program REVIGO.

We found 42 genes differentially expressed between fish from L group and H group. In the skin of the L group there were 39 genes up-regulated and 3 genes down-regulated compared to the H group of fish. In the up-regulated genes, we found 19 GO terms enriched for biological process and one for molecular functions (calcium ion binding). No GO term were enriched in down-regulated genes. The clustering of significant GO terms for biological process in up-regulated genes identified 6 GO terms (Figure 1). Within these terms are included genes related to the response to allergen, which could explain the low lice count in this group of fish.

Figure 1. Bubble graph of clustering of GO term enrichment obtained by REVIGO. Intense of color indicates the p-value. Bubble size indicates the frequency of the GO term in the underlying Gene Ontology Annotation (UniProt-GOA) database. Highly similar GO terms are linked by edges in the graph, where the line width indicates the degree of similarity.

## STATUS OF *Artemia franciscana* PRODUCTION IN KENYAN COASTAL SALTWORKS

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*Artemia franciscana* was first introduced in Kenyan solar saltworks in the mid 80's to test the feasibility of producing it in the salt ponds. The viability of producing the *Artemia* species was a success besides improving the quality and quantity of salt produced from the saltworks. Production of the brine shrimp *Artemia* in solar saltworks for aquaculture development, however started in 2010 (20 years after its introduction into the salt ponds). This initiative targeted the rural communities in coastal Kenya with the overall objective of building their capacity for *Artemia* production in existing salt production systems and application in local aquaculture initiatives to raise their socio-economic status by offering alternative livelihoods. This has been achieved by generating extra income through production of *Artemia* cysts and biomass which is crucial for the optimal local development of shrimp and fish larviculture. The initiative has helped build capacity of local institutions to develop sustainable and environmentally sound models of *Artemia* pond production and application of locally produced *Artemia* in emerging aquaculture. The local hatcheries have benefitted greatly by using the locally produced *Artemia* cysts which many have termed as a better option as compared to the imported *Artemia* cysts. However, the initiative has not lacked its fair share of challenges such as eutrophication, predation, access to sea water challenges, land tenure and unstable weather conditions with which some have been addressed. The biggest challenge is the hindrance in commercializing the industry since the seven commercial salt farms have not taken up the enterprise citing less income generated from the sale of *Artemia* cysts as compared to their core business of salt production. Six artisanal farmers have responded positively to the integrated *Artemia* production and have upscaled their farms to practice *Artemia*/fish/salt production.



## **SOME CHALLENGES AND OPPORTUNITIES IN CAPACITY BUILDING AT POST GRADUATE LEVEL IN FISHERIES AND AQUACULTURE IN EAST AFRICA**

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Africa has the youngest population in the world, with almost 200 million people between the ages of 15 and 24—a number that is expected to double by 2045 (African Economic Outlook 2015). The rising youth population especially in East Africa is increasingly better educated, and there is an unprecedented opportunity for economic and social development if the talents of this generation can be tapped. However, there is a significant gap between the skills demanded by the private sector and those supplied by Universities and informal education and training programs, including specialized technical skills, entrepreneurial/business skills, and soft skills. The knowledge and post-graduate research experience embedded in the curriculum should promote integration of sustainable development into the day-to-day fisheries and aquaculture management and strengthen academic organizations to be better able to address the globalization and sustainability challenges in fisheries and Aquaculture. In general, there is need for a much higher skill level and more systematic, private sector engagement in developing appropriate curriculum for formal and informal courses and providing opportunities for youth to get meaningful practical experience and training. One of the curriculum challenges is in repackaging how Agriculture, Fisheries and Aquaculture are taught in schools and universities. Sadly, Agriculture is widely perceived by youth as an unappealing, traditional, labor-intensive farm activity, which generates little if any profit, and not as a potentially high-profit business activity that involves a spectrum of new opportunities. We therefore need post-graduate programs that facilitate collaboration between universities with private sector associations and other partners to develop, adapt, and disseminate problem-solving innovations in Fisheries and Aquaculture.

## **GROWTH, YIELDS AND ECONOMIC BENEFIT OF NILE TILAPIA (*Oreochromis niloticus*) FED DIETS FORMULATED FROM LOCAL INGREDIENTS IN CAGES**

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Small-scale aquaculture in Africa is limited by cost of protein ingredient in fish feeds. We evaluated the suitability of replacing fishmeal with rice bran alone or rice bran in combination with atyid shrimp (*Caridina nilotica*) on growth performance and economic benefits of Nile tilapia (*Oreochromis niloticus*) culture in cages suspended over earthen ponds. The best growth and feed conversion occurred in fish fed fishmeal followed by a combination of rice bran and *C. nilotica*, while rice bran alone resulted in poorest fish growth. The best economic benefit was from fish fed a combination of rice bran and *C. nilotica*. By formulating diets using rice brain and *C. nilotica*, the cost of fish production reduced by 80%. It is thus possible to replace expensive fishmeal in the diet of *O. niloticus* using combination of cheaper rice bran and *C. nilotica* without compromising economic benefits for the small-scale fish farmer.

## A NEW APPROACH TO FEEDING LARVAE AND EARLY JUVENILES OF SANDFISH, *Holothuria scabra*, SUPPORTING SIMPLIFIED HATCHERY CULTURE METHODS

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A source of live cultured micro-algae is generally considered to be a key requirement for successful hatchery culture of sandfish, *Holothuria scabra*. However, developing and small-island nations often lack the technical resources and skilled personnel required to produce appropriate quantities of high quality live micro-algae and this is a common bottleneck. We report on recent research investigating the potential to replace live cultured micro-algae with commercially-available micro-algae concentrates during hatchery culture of sandfish.

Relative ingestion and cell wall digestion of micro-algae concentrates were assessed using epifluorescence microscopy, before the most digestible of the micro-algae were assessed for their nutritional value for auriculariae and early juvenile sandfish in subsequent growth trials. There were significant differences between the rates of survival and growth supported by the micro-algae tested (*Thalassiosira weissflogii*, TW 1200®; *Isochrysis* sp., Isochrysis1800®; and *Pavlova* sp., Pavlova1800®). TW 1200® supported consistently superior growth rates and survival of larvae, improved hyaline sphere development and superior post-larval performance. However, all micro-algae concentrates tested proved nutritious for sandfish larvae and supported normal growth and development and relatively high survival, through settlement.

Our results confirm the feasibility of using commercially available microalgae concentrates as a sole food source for hatchery culture of sandfish, and demonstrate successful hatchery culture of sandfish without using live micro-algae. Use of commercially available micro-algae concentrates as replacements for live micro-algae in sandfish hatcheries supports development of cheaper, simpler larval rearing protocols for this species.

## THE DEVELOPMENT OF A NON-LETHAL DIAGNOSTIC TOOL FOR THE DIAGNOSIS OF *Ichthyophonus hoferi*

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*Ichthyophonus hoferi* has been diagnosed at the Two Oceans Aquarium. *Ichthyophonus* is a mesomycetozoan parasite that multiplies in blood rich organs in the fish host causing a wide range of clinical signs relating to organ dysfunction. *Ichthyophonus* can be diagnosed from microscopic examination of tissue squash prep, culture or PCR. In the literature only lethal methods of diagnosis are described. The development of a non-lethal diagnostic tool for disease monitoring is vital for collections where sacrifice of specimens is not possible. Liver biopsies were obtained from (n=30) White Stumpnose (*Rhabdosargus globiceps*) comparing two surgical methods, coeliotomy (n=15) and coelioscopy, (n=15), ten fish used in a control group. Biopsy material for each fish was divided into three pieces for squash preparation examination, PCR and culture. All fish were monitored for 43 days post-surgery and blood samples drawn at two week intervals. After 43 days fish were euthanized for full examination of the liver, kidney, spleen and heart allowing correct assignment to one of two groups; *Ichthyophonus* infected fish and non-infected fish. PCR and culture of liver tissue was also performed. Preliminary results show a 64% sensitivity of the wet mount biopsy and a 38% sensitivity of biopsy in culture with a 100% specificity for both. Wet mount and culture of the biopsy showed a sensitivity of 81%. Final post mortem on all organs showed 25 fish to be positive for *Ichthyophonus*. 5 fish were negative for *Ichthyophonus* in all diagnoses. Coelioscopy was less invasive and caused fewer organ adhesions than coeliotomy.

## C1R AND C1S PROTEASES FROM *Sebastes schlegelii*; MOLECULAR AND TRANSCRIPTIONAL ANALYSIS

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C1r and C1s are serine proteases responsible for activating the classical and lectin complement pathways to initiate the complement cascade, which plays a crucial role in eliminating microbial infections. In this study cDNA sequences of C1r and C1s were identified from black rockfish transcriptome database and designated as SsC1r and SsC1s, respectively. Identified cDNA sequences of SsC1r and SsC1s were 2348 bp and 2808bp long with open reading frames (ORF) of 2112 bp and 2082 bp, respectively. They encoded proteins with 704 and 694 amino acids. In domain analysis, two CUB domains, two CCP domains and a Trypsin-like serine protease domains were observed in both sequences. Multiple sequence alignment, with encoded amino acid sequences, was conducted separately for these two genes with orthologs from other species. High conservation among species throughout the sequence was observed for both. A phylogenetic tree was constructed, using neighbor-joining method, to assess the evolutionary relationship, with C1r and C1s amino acid sequences from several species. C1r and C1s formed two separate clusters, which further assembled into groups of related organisms. SsC1r and SsC1s joined with the respective teleostean clusters. Transcriptional analysis showed the highest mRNA expression levels in liver under normal physiological conditions. Significantly upregulated expression in spleen was observed after injection under the pathogenic stress by lipopolysaccharide polyinosinic: polycytidylic acid and *Streptococcus iniae* at 6 to 24 hours after challenge. The serine protease domain of SsC1r and SsC1s were cloned and expressed in *Escherichia coli*. Functional assays are underway to prove their protease activity. Results from present study suggests the possible involvement of SsC1r and SsC1s in rockfish immune system.

## PRECISION BOMBING OF THE WHITE SPOT SYNDROME VIRUS TRANSCRIPTOME

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In the siRNA pathway, double stranded RNA (dsRNA) is cleaved and processed by a host gene apparatus into short siRNA molecules associating with the RNA-induced silencing complex (RISC). In RNA interference (RNAi), a mechanism targeting replication of viruses and mobile genetic elements, RISC cleaves mRNA transcripts with sequence complementarity to the loaded siRNA and inhibits translation of encoded proteins.

White spot syndrome virus (WSSV), a large double-stranded DNA virus belonging to the family *Nimaviridae*, is the major viral disease problem in shrimp aquaculture worldwide. WSSV has a wide host range of decapod crustaceans ranging from penaeid shrimp in tropical regions to lobsters in temperate regions. Several studies have demonstrated that injection of dsRNA homologous to mRNA for the viral envelope protein VP28 can induce protection in shrimp against WSSV through RNAi. Here we compare the RNAi response in white leg shrimp (*Penaeus vannamei*) injected with VP28-specific dsRNAs to that of non-treated shrimp and shrimp injected with dsRNAs targeting a nonspecific control, the green fluorescent protein (GFP). Nearly complete protection against infection was achieved in shrimp injected with VP28 dsRNAs, while the nonspecific GFP controls performed only slightly better than non-treated shrimp.

RT-qPCR gene expression- and RNA sequencing analyses have been performed on samples collected during this WSSV challenge trial, and the results demonstrated, both in terms of host gene expression pattern and characteristics of the WSSV specific siRNA population, how the highly protective RNAi response in shrimp treated with WSSV VP28 specific dsRNA differs from control shrimp receiving either nonspecific GFP dsRNA or no treatment through a PBS injection. A relatively modest induction of the RNAi siRNA-pathway leads to a highly intensive and focused siRNA population in response to a WSSV VP28 specific dsRNA treatment, which resulted in the inhibition of WSSV replication and WSSV-induced shrimp mortality.

# UNDERWATER FISH VOLUME ESTIMATION USING AN OPEN CAVITY HELMHOLTZ RESONATOR

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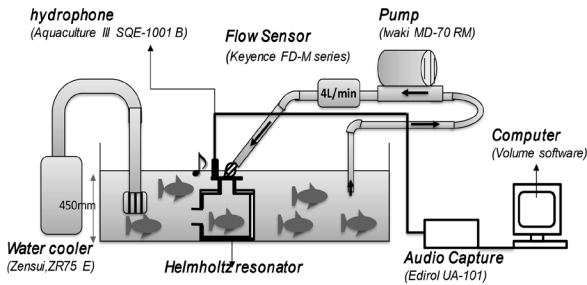
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In Aquaculture, fish are manually sorted on commercial and leisure vessels based on weight and species. To do this length measurements are taken on the carrier vessel with one person measuring and the other jotting down the measurements. This is laborious and time consuming. Therefore, there is a need for a fast, reliable, non-invasive method to estimate the volume of fish.

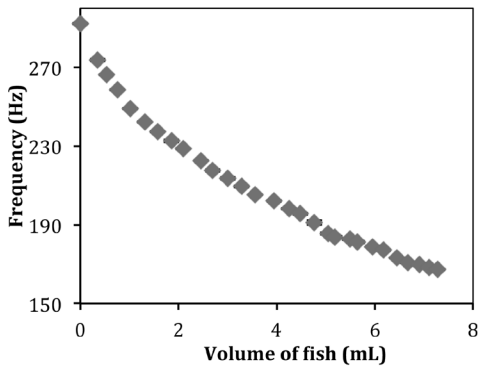
We used a technique known as Helmholtz resonance to estimate volume of aquatic products. While this technique has already been used in air, in this study we examine the principles and physics of a multi-neck Helmholtz resonator underwater, where the target measurement object could be either lighter or denser than that of the water medium. To validate these derived principles, air was experimentally introduced into the resonator as a less dense medium, which could also model the behavior of a swim bladder (hydrostatic organ for fish). Finally, as proof of concept, we measured the Helmholtz resonance of a model fish of varying volume to demonstrate the possibility of underwater volume measurement using a double neck Helmholtz resonator, as shown in Figure 1.

Resonant frequency decreased as the volume of air increased in both closed and open cavities of the resonator. This was because of the bulk modulus of air being less than that of water. With increased volume of fish in both cavities, there was damping of the resonant frequency (Figure 2). This was attributed to losses due to viscosity, caused by swim bladder in the fish.

This direct relationship between fish volume and resonance frequency has the potential to be used for non-invasive measurement of fish volume.



**Figure 1** General experimental set up with a resonator



**Figure 2** Frequency against volume of fish in a closed cavity of a resonator



# CULTURE TRIAL OF THE GIANT FRESHWATER PRAWN, *Macrobranchium vollenhovenii*, HERKLOTS, 1857 AROUND THE FUTO AXIS OF OTAMIRI RIVER, NIGERIA

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Nigeria is blessed with abundant shellfish resources of which the giant fresh water prawn, *Macrobranchium vollenhovenii* is considered the best candidate for aquaculture based on fast growth and good table size. Commercial production will reduce the present deficit of 1.8 million metric tons in domestic fish demand and substantially conserve the current fish import bill of USD 600 million in foreign exchange.

Culture trial of 7488 juveniles of the giant freshwater prawn, *Macrobranchium vollenhovenii* Herklots, 1857 was carried out for 180 days in concrete tanks to evaluate adaptability, survival, feed utilization and growth performance in captivity. Twelve experimental ponds each measuring 10m x 5m x 1.0m were used for the experiment between march and August 2016. The prawns were stocked two weeks after conditioning the tanks with agricultural lime at the rate of 2279kg ha<sup>-1</sup> and poultry manure at the rate of 114kg ha<sup>-1</sup>. Four experimental diets tested were designated as T<sub>1</sub>: powdered broiler starter, T<sub>2</sub>: commercial brine shrimp; T<sub>3</sub>: combination of broiler starter and brine shrimp and T<sub>4</sub>: MAFES diet formula. Diets were randomly assigned to the four treatment tanks and replicated three times in a 4 x 3 = 12 experimental pond units as a completely random experiment (CRD). Each tank was stocked with 624 juvenile prawns with mean total length of 1.5cm and body weight of 10.0 grams. Prawns were fed at 5% body weight and two times daily at 0600 and 1800 hours. Sampling for growth was carried out bi-weekly while water quality was tested weekly. Results were statistically evaluated with one way analysis of variance (Anova). Water quality of the four tanks were not significantly different (P > 0.05) from one another throughout the culture period and conformed to recommended tolerable limits in culture ponds. Growth performance (Table 1) was best in diet T<sub>4</sub> (the MAFES formula), with a survival rate of 75%, mean weight gain (MWG) of 65.0g, relative growth rate (RGR) of 650%, specific growth rate (SGR) of 1.12%, average daily growth (ADG) of 0.36g day<sup>-1</sup> and food conversion ratio (FCR) of 1.44. The above values differed quite significantly (P < 0.05) from growth responses of other diets. The computed gross ratio (GR) of 0.62 suggests that prawn culture is a highly lucrative business.

Table 1: Growth response of experimental prawns to dietary treatments during 180 days culture trial.

| Growth Parameter                | Treatment diets   |                   |                   |                   |
|---------------------------------|-------------------|-------------------|-------------------|-------------------|
|                                 | T <sub>1</sub>    | T <sub>2</sub>    | T <sub>3</sub>    | T <sub>4</sub>    |
| Initial mean weight (g)         | 10.0 <sup>a</sup> | 10.0 <sup>a</sup> | 10.0 <sup>a</sup> | 10.0 <sup>a</sup> |
| Final mean weight of (g)        | 45.0 <sup>a</sup> | 35.0 <sup>b</sup> | 50.5 <sup>a</sup> | 75.0 <sup>c</sup> |
| Mean weight gain, MWG (g)       | 35.0 <sup>a</sup> | 25.0 <sup>b</sup> | 40.5 <sup>a</sup> | 65.0 <sup>c</sup> |
| Number of Prawns stocked        | 18 72             | 1872              | 18.72             | 1872 <sup>a</sup> |
| Number that survived at harvest | 1311 <sup>a</sup> | 1498 <sup>b</sup> | 1424 <sup>c</sup> | 1404 <sup>c</sup> |
| Rearing period (days)           | 180               | 180 <sup>b</sup>  | 180               | 180               |
| Survival rate (%)               | 70 <sup>a</sup>   | 80 <sup>a</sup>   | 75 <sup>a</sup>   | 75 <sup>a</sup>   |
| Relative growth Rate, RGR (%)   | 350 <sup>a</sup>  | 250 <sup>b</sup>  | 405 <sup>b</sup>  | 650 <sup>c</sup>  |
| Specific growth rate, SGR (%)   | 0.84 <sup>a</sup> | 6.98 <sup>b</sup> | 8.98 <sup>c</sup> | 1.12 <sup>d</sup> |
| Average Daily growth, ADG (g)   | 0.19 <sup>a</sup> | 0.14 <sup>b</sup> | 0.23 <sup>a</sup> | 0.36 <sup>c</sup> |
| Food conversion ratio, FCR      | 2.70 <sup>a</sup> | 3.74 <sup>b</sup> | 2.3 <sup>a</sup>  | 1.44 <sup>c</sup> |

abc: mean values in the same row with different superscripts are significantly different (P < 0.05).

## THE TRAINING APPROACHES IN AQUACULTURE: THE NIGERIAN EXPERIENCE

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The practice of aquaculture has developed as a profession in recent years and this has attracted the entrance of many folks. Unfortunately, aquaculture has almost become an all comers affair with several indicators pointing to lack of the needed expertise. As a result of this, different approaches have been adopted to boost the needed competence in aquaculture towards better practice in order to achieve the expected increase in fish protein intake and food security.

Different approaches have been followed towards the training of professional and “associate” aquaculture practitioners. This was aimed at achieving best practices particularly in an era of global competition. One challenge encountered is the very low enrolment of candidates who intend to study aquaculture in the tertiary education sector. The approach of adopted schools has proved to be a useful tool to reduce the apathy that has been recorded. This also necessitated the need to devise further motivational and hands on approaches. The utilization of Students’ Industrial Works Experience Scheme

(SIWES) was found to be a useful tool in this direction. In addition, the approach of adopted farms and villages has played great roles in the establishment of homestead aquaculture practice which resulted in the creation of small holder farmers. The different approaches that have been utilized to boost fish productivity particularly in local communities have given rise to increased protein intake particularly in the rural areas. These approaches are aimed at developing a new generation of aquaculture experts that will open new frontiers for economic growth.

## INFLUENCE OF FEED TYPE AND AGE OF BROODSTOCK ON REPRODUCTIVE PERFORMANCES OF THE CATFISH, *Heterobranchus longifilis*

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The study was conducted to investigate the influence of feed type and age of broodstock on the reproductive performance of *Heterobranchus longifilis*. Males and females of 6 month old, 1½ year old and 2½ year old *H. longifilis* were subjected to 42% crude protein level of commercial and farm made feeds for one year. Artificial breeding was carried out using milt from the various age groups to fertilize eggs of the corresponding age groups.

Percentage fertilization, hatchability, survival and production success of fry performance were assessed and compared. Feed type had significant effects on egg weight, number of eggs per gram ovary weight, absolute fecundity, relative fecundity, spermatocrit and gonadosomatic index (GSI) but was not significant for egg diameter. Three and half (3) year old females had 391.38 g weight of eggs, 499.74 number of eggs per gram ovary weight, 19,558 absolute fecundity and 20.23 gonadosomatic index which were significantly higher than the other two age groups (1½ and 2½). On the average, stages of gonad development were significantly increased by feed type and age. Percentage of immature eggs produced by broodstock fed farm made feed was significantly higher than those produced by broodstock fed commercial feed while the immature eggs decreased significantly with age. The broodstock fed the commercial feed recorded relatively minor increase in milt volume (3.77 ml) above the other group fed with farm made feed (3.75 ml) but showed no significant difference. However, broodstock fed commercial feed had 68.38 cm/sec sperm motility which was significantly higher than 64.49 cm/sec obtained from those fed with farm made feed. Generally, milt quality considered in this study significantly increased with age except spermatocrit which decreased with age. Feed type had no significant effect on percentage fertilization, hatchability and fry survival of *Heterobranchus longifilis* but a significant effect was observed in fry production success. However all these reproductive performances increased with the age of the broodstock.

A NEW DESICCATION-TOLERANT AND PATHOGEN FREE LIVE FEED FOR SHRIMP AND FISH AQUACULTURE

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The sustainability of the shrimp and fish aquaculture industry is threatened by the strong dependence of hatcheries on the live feed *Artemia*, with its production levels varying subject to seasonal weather and a resulting price volatility. The company e-nema (Germany) has developed a new dehydrated live feed that can be stored and rehydrated/reactivated on demand. The new pathogen free feed *Panagrolaimus* sp. is a nematode species which is mass produced in sterile conditions in bioreactors at constant yields with easily available land bound feedstock. It can be stored as long as the dormant eggs of *Artemia*; it is easy to use and performs better than inert diets. Reactivation is initiated by rehydrating the product in water for only 30 min at room temperature with no further need for specialized infrastructure or labour. This alternative live feed is one solution to satisfy the demand and to further stabilize the sustainability of this industry.

A feeding trial was carried out on white legs shrimp (*Litopenaeus vannamei*) postlarvae (PL) fed three dietary treatments for 10 days, from PL13 to PL22 in a recirculated aquaculture system (RAS): 1) *Artemia* and an artificial diet, 2) Nematodes and an artificial diet, and 3) artificial diet without live feed. In a second part, shrimp larvae were further cultivated 11 days on the artificial diet from PL23 to PL33.

Nematodes produced larval performances equal to those of *Artemia* at the end of the live feed period at PL23 (15.5 mm and 46.8 mg in average) and at PL33 (142.3 mg and 88.9% final survival, in average) (Table 1). Nematodes produced PL23 with a narrower spectrum of lengths than *Artemia*, which is better for economic reasons (Figure 1).

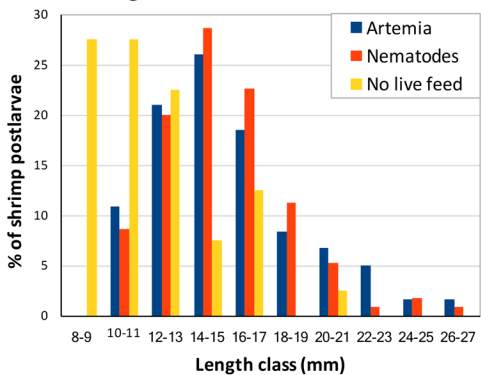
Shrimp fed on the artificial diet only were smaller at PL23 (11.9 mm, 26.0 mg) and at PL33 (114.0 mg), showing the benefit of live feeds.

For the first time a nematodes species mass produced in sterile conditions and stored desiccated was successfully reactivated as a live feed to replace 100% *Artemia*, during the nursery of shrimp PLs in RAS at precommercial level.

Table 1 Growth parameters and survival

|      |              | Dietary treatment |             |              |
|------|--------------|-------------------|-------------|--------------|
|      |              | Artemia           | Nematode    | No live feed |
| PL23 | Length (mm)  | 15.6 ± 0.0        | 15.4 ± 0.5  | 11.9 ± n/a   |
|      | Weight (mg)  | 45.0 ± 1.4        | 48.5 ± 7.8  | 26.0 ± n/a   |
| PL33 | Survival (%) | 82.8 ± 8.5        | 95.1 ± 5.4  | 90.6 ± n/a   |
|      | Weight (mg)  | 157.0 ± 32.5      | 127.5 ± 3.5 | 114 ± n/a    |

Figure 1 Length distribution at PL23





## REARING PERFORMANCE OF FLOATING HAPA BAG NETS FOR JUVENILE SANDFISH *Holothuria scabra* IN CENTRAL PHILIPPINES

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Global sea cucumber populations are declining because of overfishing. Hatchery production of sea cucumbers, particularly of the tropical sandfish *Holothuria scabra* have been progressing in the past decade but the corresponding improvements in nursery and grow-out technology have been slow. Various nursery systems for rearing of sandfish has been developed and modified through the years to optimize growth performance and survivorship. Sea-based nurseries, particularly the floating hapa nursery systems have shown great production potential and practical applications especially in the Philippines and the West Pacific regions. To evaluate rearing performance of floating hapa bag net for juvenile sandfish, various experiments were conducted by SEAFDEC/AQD in Iloilo and Guimaras, central Philippines. Performance indicators such as growth, survival, and optimal stocking densities were assessed using 40-45 d old post-metamorphic juveniles (~5mm, ~0.03g) produced in the hatchery. Floating hapa bag nets were made from fine-mesh (1 mm) PE net fabricated to a dimension of 1×2×1 m (Length×Width×Depth) and fitted on floating PVC pipes to be suspended from the water surface (Fig. 1). Experiments were conducted at the Igang Marine Station (IMS) of SEAFDEC/AQD located in Guimaras Island, central Philippines.

The general trend in survival and growth of sandfish juveniles with different initial stocking densities after a 30-d culture duration is presented in Table 1. During a good season with no heavy rains, high survival (>80%) and good growth (>2 g) can be achieved at densities of <700 individuals per hapa without supplemental feeding. Sandfish only fed on naturally accumulating algal biofilm on the nets. Results indicated that growth and survival is significantly influenced by stocking density, where average growth rates increase as density decreases. However, other variables such as food composition and abundance, seasonality and competition/predation are among the challenges that must be considered for future studies. With an understanding of these site-specific factors, nursery rearing can be programmed to optimize production potential of sandfish juveniles in sea-based floating hapas.



FIG 1. Sandfish floating nursery at SEAFDEC/AQD Philippines

TABLE 1. Survival and growth of sandfish juveniles in floating hapas with different stocking densities after 1 mo rearing

| Stocking density<br>(Individuals/hapa) | Survival / Individuals | Ave Weight |
|--|------------------------|------------|
| 250                                    | 95% / 237              | 3.2 g      |
| 500                                    | 90% / 450              | 2.3 g      |
| 700                                    | 85% / 595              | 1.8 g      |
| 1000                                   | 75% / 750              | 1.6 g      |
| 2000                                   | 68% / 1360             | 0.8 g      |

## AQUA-SPARK: INVESTING IN THE FUTURE OF AQUACULTURE

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Aqua-Spark is an investment fund with a focus on sustainable aquaculture businesses around the world. The small-to-medium enterprises (SMEs) we invest in are working toward the production of safe, accessible aquatic life, such as fish, shellfish and plants, in ways that do not harm our oceans. Our investors value the fact that each investment aims to create triple impact—specifically, each investment is chosen for its potential to generate significant financial returns while also activating positive environmental and social outcomes.

Aqua-Spark believes that committing to a long-term vision is the way to realize effective and lasting impact results. When it comes to investments, we do not seek quick exits; instead, we look for entrepreneurs who strive to build and scale toward the future, who see their business as major economic opportunities that can also help solve looming environmental and food security challenges over the long-term. As an essential of each investee, Aqua-Spark seeks sustainability.

Specifically, our fund chooses SMEs that meet the following three principles of sustainability;

- Economic sustainability—the business generates enough cash to be able to reinvest in its own growth and provide an equitable return to its stakeholders;
- Environmental sustainability—the business sets high standards of environmental management and seeks to maintain or enhance ecosystem services; and
- Social sustainability—the business creates jobs, reduces poverty, empowers the underserved, and helps to strengthen communities;

Not all investments will meet all principles equally, but all will strive to. Aqua-spark will not invest in any businesses that makes anybody's life worse, discriminates against any person based on their gender, race, national origin and religion, or that causes irreversible changes in the environment.

Our investments start at \$250,000. We do not seek controlling stakes in our investments. We prefer to be a minority investor holding between 20-49%, alongside other investors. Every business we invest in is a reflection of our trust in the entrepreneur to lead their companies' development and growth in the most effective way.

## AQUA-SPARK: INVESTING IN THE FUTURE OF AQUACULTURE

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**A COMPARATIVE STUDY OF GROWTH RATES AND YIELD OF THE SEAWEED, *Kappaphycus alvarezii* (Doty), USING DIFFERENT SEEDLING DENSITIES AND FARMING METHODS IN SOUTH COAST, KENYA**

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The study aimed to generate information that will contribute to development of alternative livelihoods as a policy to uplift the socio- economic status of small-scale farmers at Kibuyuni and Gazi . The study identified ecological indicators based on the objectives of sustainability of harvests, farming methods, seedling densities site characteristics and seasonal variations in growth rates. Data analyzed were sourced from on-project fieldwork. Trends in growth rates and yield of *Kappaphycus alvarezii* (2014-2015) from the two sites in the south coast were analyzed using canonical correspondence analysis, 3 way Anova and Anderson- Darling test . Growth rate was done fortnightly and hydrographic parameters on monthly basis at two sampling stations. The Gazi Bay was influenced by variable river discharges and mainly affected by primary and secondary wet seasons in March–June and August–November, respectively. The temperature varied with a pronounced peak in both the primary and secondary rainy seasons..

The medium seedling density (100g) from the raft type of farming accounted for 72.06% (897.9 g/m and the lowest seedling density (50g) accounted 40 % yield in Gazi Bay while at Kibuyuni the highest yields was from the highest density 60% yield. Temporally, the mean growth rate of *Kappaphycus alvarezii* and biomass in the off bottom method was lower during the primary wet season than during the secondary rainy periods while spatially, the mean biomass was higher in the middle zone of the bay systems. Gazi Bay showed a strong correspondence with turbidity and pH, whereas Kibuyuni strongly corresponded with salinity. Biodiversity and conservation based indicators adopted from the Indiseas program ([www.Indiseas.org](http://www.Indiseas.org)) showed the Gazi Bay ecosystem to be ecologically suitable in terms of production output, lower herbivory incidences, lower *ice ice* occurrences and high sedimentation as well as high ammonia inputs. The study recommended adoption of the studied ecological indicators and tools as means of evaluating and monitoring the two sites resources and ecosystem status.

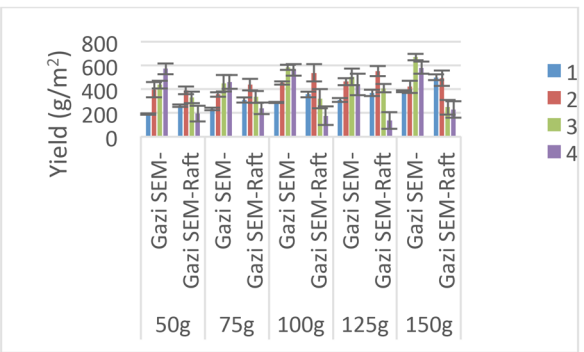


Fig 1. Yield of *K. alvarezii* in Kibuyuni on 45th day of culture period under different seed densities

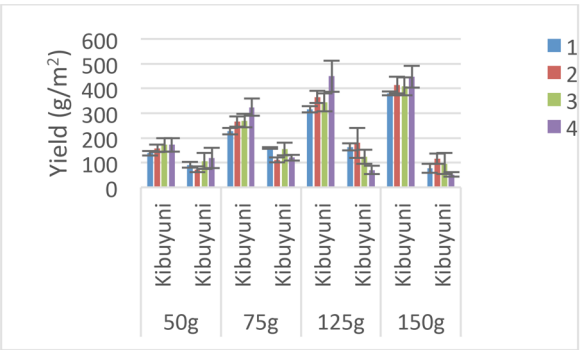


Fig 2. Yield of *K. alvarezii* in Kibuyuni on 45th day of culture period under different seed densities .

## ACUTE TOXICITY OF NPK 15:15:15 FERTILIZER TO AFRICAN CATFISH AND THE EFFECT ON BLOOD, LIVER ENZYMES AND HISTOLOGY OF THE GILLS AND LIVER

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N.P.K 15.15.15 fertilizer is in aquaculture to boost plankton production. However, introduction of excess fertilizer into aquaculture environment has physiological and biochemical defects in fishes (Sarkar and Rashid 2012). Inside the water they attach on aquatic vegetation and substrates where fishes pick them up during feeding. Therefore this study examined the extent of physiological and biochemical alterations that NPK 15:15:15 fertilizer can cause to African catfish, *Clarias gariepinu*, with a view to determining the safe concentration of the fertilizer that can be used in aquaculture water.

Range finding tests were conducted using varied concentrations of the NPK fertilizer as a guide to determining the 96h acute toxicity of the fertilizer to African catfish ( $4.84 \pm 0.2g$ ) at different concentrations of 0g/l, 4.2g/l, 4.4g/l, 4.6g/l, and 4.8g/l, respectively for treatments 1-5. After 96h the percentage mortality was converted into probit and the concentrations into logit to calculate the 96h  $LC_{50}$ . Survived fish from each treatment group were assayed for blood profile and liver enzyme analyses using standard methods. Dead fish also in determining the histological effect of the fertilizer on the gill and liver of the fish.

Results indicated the 96h  $LC_{50}$  of NPK 15:15:15 to African catfish ( $4.84 \pm 0.2g$ ) as 4.74g/l of water. Fish exposed to NPK fertilizer showed erratic swimming movements, restlessness, skin discoloration, and hyperactivities. While activities of AST, ALT, ALP and GGT increased with the concentration of the NPK, RBC, Hb, PCV, MCH and MCHC decreased, and WBC increased. Histological examination revealed alterations in gill architecture, and severe vacuolation and necrosis of the liver cells.

## EFFECT OF STOCKING DENSITY AND DIETARY PROTEIN ON GROWTH AND SPAWNING PERFORMANCE OF SINGIDIA TILAPIA (*Oreochromis esculentus*) BROODFISH-KENYA

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Singidia tilapia (*Oreochromis esculentus*) endemic to Lake Victoria and once the most important commercial fish species in the Lake is now enlisted in the World Conservation Union Redbook (IUCN) of endangered species as critically endangered. To rescue this highly valued fish from total extinction, urgent conservational measures including captive propagation and reintroduction are required.

A 126 days study was set out to investigate the effect of *O. esculentus* broodstock stocking density on seed production. In a 2x3 factorial design, two different *O. esculentus* broodstock feed (25% crude protein and 35% crude protein) were combined with three different broodstock densities (4, 8 and 12 fish/ m<sup>2</sup>) to obtain six experimental treatments. Four replicate groups of broodfish were stocked in spawning hapas at a ratio of one male to one female (1 ♂ : 1 ♀). The experimental design is shown in Table 1. Broodstock were fed with the experimental diet at a feeding rate of 3% of total biomass in each experimental hapa (six days/week).

After the 126 days, *O. esculentus* female broodfish stocked at 4 fish/m<sup>2</sup> and fed at 35% dietary protein recorded significantly ( $p < 0.05$ ) higher average final weight and seed output with those stocked at density 12 and fed with 25% CP recording the least. No significant difference in Seed/g female was recorded between broodfish fed with 35% CP at the highest stocking density (12 fish/m<sup>2</sup>) and 25% CP at the lowest stocking density (4 fish m<sup>2</sup>) -Table 2.

This study showed that stocking density and crude protein level significantly affect the natural spawning success in mature *O. esculentus* broodstock. The lowest stocking density (4 fish m<sup>2</sup>) resulted in high seed production and was more profitable in terms of seed output and recommends to stock *Oreochromis esculentus* brooders in spawning hapas at a stocking density of 4 fish/m<sup>2</sup> and feed them with 35% crude protein diet.

TABLE 1. The experimental design

| Treatment | Protein level | Broodstock density                     |
|-----------|---------------|--|
| T1        | 25% CP        | 4 (2 ♂ : 2 ♀) fish / m <sup>2</sup> .  |
| T2        | 25% CP        | 8 (4 ♂ : 4 ♀) fish / m <sup>2</sup>    |
| T3        | 25% CP        | 12 (6 ♂ : 6 ♀) fish / m <sup>2</sup>   |
| T4        | 35% CP        | 4 (2 ♂ : 2 ♀) fish / m <sup>2</sup>    |
| T5        | 35% CP        | 8 (4 ♂ : 4 ♀) fish / m <sup>2</sup> .  |
| T6        | 35% CP        | 12 (6 ♂ : 6 ♀) fish / m <sup>2</sup> . |

TABLE 2: Effect of protein level and stocking density on growth and seed production

| Treatment |       | IAW                | AFW                | S/f/h               | S/g/f               |
|-----------|-------|--------------------|--------------------|---------------------|---------------------|
| CP        | Dnt y |                    |                    |                     |                     |
| 25 %      | 4     | 125.5 <sup>a</sup> | 170.5 <sup>a</sup> | 494.1 <sup>cb</sup> | 15.9 <sup>cb</sup>  |
| 25 %      | 8     | 123.5 <sup>a</sup> | 166.3 <sup>a</sup> | 423.4 <sup>cd</sup> | 13.8 <sup>5cd</sup> |
| 25 %      | 12    | 123.5 <sup>a</sup> | 164.3 <sup>a</sup> | 381.3 <sup>d</sup>  | 12.6 <sup>5d</sup>  |
| 35 %      | 4     | 125.5 <sup>a</sup> | 177.4 <sup>a</sup> | 969.9 <sup>a</sup>  | 30.3 <sup>7a</sup>  |
| 35 %      | 8     | 124.0 <sup>a</sup> | 175.5 <sup>a</sup> | 887.1 <sup>a</sup>  | 28.3 <sup>8a</sup>  |
| 35 %      | 12    | 126.2 <sup>a</sup> | 170.5 <sup>a</sup> | 586.7 <sup>b</sup>  | 18.9 <sup>3c</sup>  |

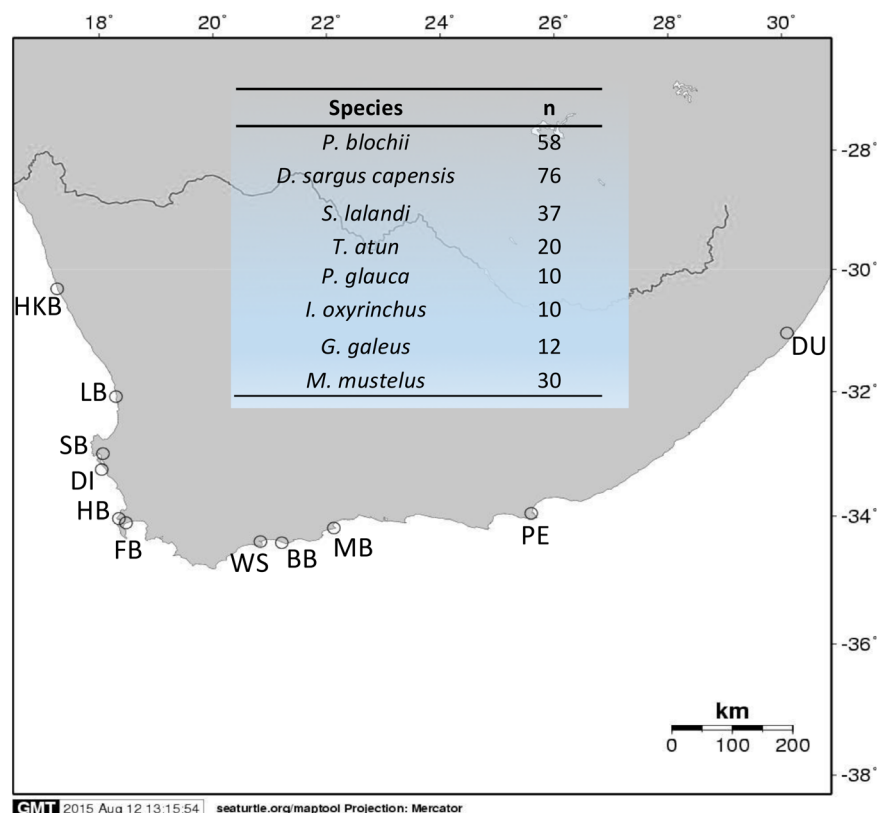
## METALS IN SOUTH AFRICAN MARINE FISH SPECIES - A GEOGRAPHICAL PERSPECTIVE

<sup>1</sup>O'Neill, B., <sup>1</sup>Bosch, A., <sup>3</sup>Firth, C., <sup>2</sup>Sigge, G. O., <sup>2</sup>Kerwath, S. E & <sup>2</sup>Hoffman, L. C.

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Marine fish offer a valuable source of nutrition, containing high quality protein and omega fatty acids which when consumed regularly can offer a wealth of health benefits to consumers. In spite of these benefits, fish have also been shown to accumulate toxic molecules such as Persistent Organic Pollutants and metals to levels which can be damaging to humans if consumed in sufficiently high quantities. Such toxic molecules are known to bio-accumulate up the food chain; therefore, concentrations tend to increase from lower to high trophic level species: herbivore - carnivore - top predators. Although many foodstuffs contain metal contaminants above regulatory limits marine fish tend to have some of the highest levels in particular arsenic, cadmium, mercury and lead.

Monitoring the metal levels of commonly consumed fish at different trophic levels is thus essential to ensure safe and healthy products for consumers. The current study determined the concentrations of 16 metals from 8 fish species over 11 coastal locations along the South African coastline (Fig.1). This study will not only inform fishers, processing companies and consumers but also fish farmers with an interest in farming any of the eight species studied.



**Figure 7.1** Locations of sampling sites along the South African coastline where HKB-Hondeklip Bay; LB-Lamberts Bay; SB-Saldanha Bay; DI-Dasses Island; HB-Houte Bay; FB-False Bay; WS-Witsand; BB-Blombos; MB-Mossel Bay; PE-Port Elizabeth; DU-Durban.

## ECONOMIC PROFITABILITY OF SMALL-SCALE INTEGRATED FISH-RICE-POULTRY FARMS IN CAMEROON

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Increasing population as is the case in most African countries equates to increasing demand for food. A sustainable agricultural system that can meet such an increase in demand by maximally utilizing available resources and minimizing wastage is therefore of great economic benefit. Integrated aquaculture which is a diversified and coordinated way of farming with fish as the main target along with livestock or crop production offers much promise in this regard. There are therefore increasing efforts from diverse national and international stakeholders to promote integrated aquaculture for its merit in meeting the triple needs of food production, social and economic relevance and environmental stability.

This research studied 120 aquaculture producers in the Southwest and Northwest regions of Cameroon under the Multi-Donor Trust Fund (MDTF)/World Bank grant to some African Universities. The hypothesis is that socioeconomic parameters influence farmers' selection of an enterprise mix amongst fish-rice-poultry that will maximize revenue. A revenue equation is estimated by imposing a Cobb-Douglas functional form. About 35% of farmers practiced integrated systems, and for those that did not practice in situ –integrated systems, about 65% undertook these different enterprises at different times within the same calendar year. The combination of poultry with fish-rice enterprises is practiced as a means of increasing farm income through better resource utilization. The systems are overall profitable. When integration were undertaken for two systems either as fish cum rice, fish cum poultry or rice cum poultry, farmers realized annual net returns of 428,630 FCFA (US\$ 857); 785,650 FCFA (US\$ 1,571) and 865,990 FCFA (US\$ 1,732), respectively. The highest profits of 965,270 FCFA (US\$ 1,931) were obtained in the complete fish-rice-poultry system. Econometric estimates of the revenue function reveal positive and direct relationship of farm revenue with socioeconomic parameters such as plot size, labour, credit, education, land tenure, access to extension and enterprise integration. For dissemination and adoption of this technology there is a need for training in pond management and system product marketing.

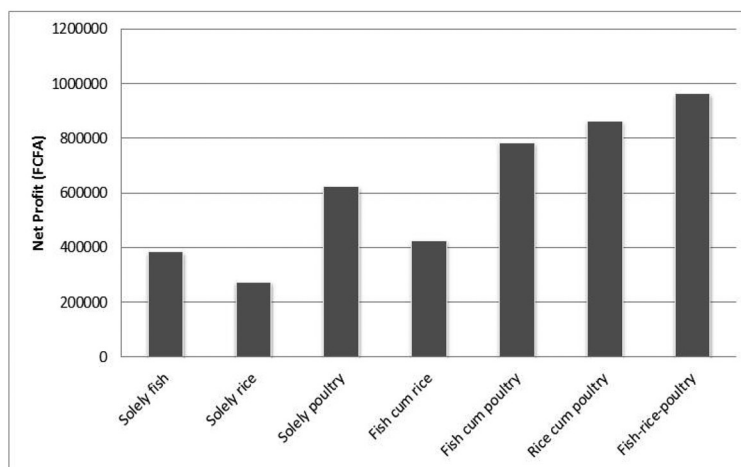


Figure 1: Comparative profitability of different enterprises and systems (per farmer)

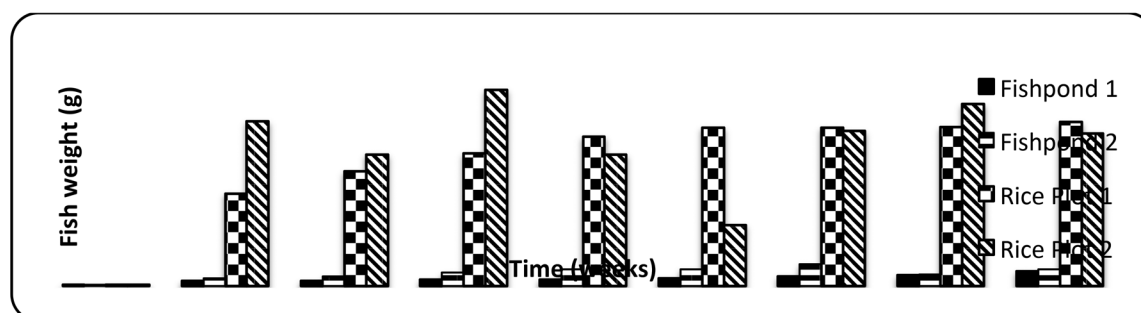
## ENHANCING FISH PRODUCTION IN CAMEROON: INTEGRATED FISH-RICE FARMING VERSUS EARTHEN POND CULTURE IN BUEA, SW CAMEROON

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Integrated fish-rice farming (FRF) is a possible solution to the problem of high costs of fish feeds in aquaculture in Cameroon. For 12 weeks at the University of Buea/ CORAF Integrated research farm, the growth of *Clarias gariepinus* fish species was investigated in concurrent extensive culture with rice (*Oryza sativa*, L.) in two rice plots (Rice Plot 1 and Rice Plot 2) and in two earthen ponds (Fish pond1 and Fish pond2). The fish were fed compounded diet at 5% body weight. Physico-chemical water quality parameters were monitored in all the culture sites. Mean fish weight gain was significantly higher ( $P<0.01$ ) in the rice plots ( $329.21\pm44.35\text{g}$ ) than in the earthen ponds ( $30.01\pm2.84\text{g}$ ). Apart from low mean dissolved oxygen values in the Rice Plot 1 ( $3.14\pm1.19\text{ mg/L}$ ) and Rice Plot 2 ( $2.18\pm0.23\text{ mg/L}$ ), mean values for physico-chemical water quality parameters were within recommended ranges for *C. gariepinus* growth in the four culture sites. These results show that fish grew faster in the FRF than in conventional earthen ponds. It is therefore recommended as a better practice suitable for adoption by local farmers.



**Figure 1:** Growth in weight of *Clarias gariepinus* at the fish culture sites in the CORAF/WECARD adaptive research integrated fish farm, University of Buea, Cameroon from September to November, 2014

**Table 1.** Mean values of growth indices of *Clarias gariepinus* in the fish culture sites during study period

| Growth Indices (N=17)             | Fishpond 1<br>Mean $\pm$ SE   | Fishpond 2<br>Mean $\pm$ SE   | Rice Plot 1<br>Mean $\pm$ SE    | Rice Plot 2<br>Mean $\pm$ SE    | ANOVA  |
|-----------------------------------|-------------------------------|-------------------------------|---------------------------------|---------------------------------|--------|
| Initial mean total length (cm)    | 6.897 $\pm$ 0.40 <sup>a</sup> | 6.90 $\pm$ 0.40 <sup>a</sup>  | 6.90 $\pm$ 0.28 <sup>a</sup>    | 6.88 $\pm$ 0.36 <sup>a</sup>    | P>0.05 |
| Final mean total length (cm)      | 16.30 $\pm$ 0.98 <sup>a</sup> | 17.50 $\pm$ 0.99 <sup>a</sup> | 34.50 $\pm$ 1.02 <sup>b</sup>   | 29.40 $\pm$ 2.02 <sup>c</sup>   | P<0.01 |
| Initial mean weight (g)           | 2.85 $\pm$ 0.05 <sup>a</sup>  | 2.85 $\pm$ 0.05 <sup>a</sup>  | 2.85 $\pm$ 0.06 <sup>a</sup>    | 2.87 $\pm$ 0.06 <sup>a</sup>    | P>0.05 |
| Final mean weight (g)             | 30.90 $\pm$ 1.93 <sup>a</sup> | 34.81 $\pm$ 3.85 <sup>b</sup> | 343.80 $\pm$ 41.38 <sup>c</sup> | 320.31 $\pm$ 47.53 <sup>d</sup> | P<0.01 |
| Weight gain (g)                   | 28.05 $\pm$ 1.88 <sup>a</sup> | 31.96 $\pm$ 3.79 <sup>b</sup> | 340.95 $\pm$ 41.32 <sup>c</sup> | 317.46 $\pm$ 47.47 <sup>d</sup> | P<0.01 |
| Mean weight gain (g)              | 30.01 $\pm$ 2.84 <sup>x</sup> |                               | 329.21 $\pm$ 44.35 <sup>y</sup> |                                 |        |
| Weight gain (%)                   | 985.96 <sup>a</sup>           | 1121.40 <sup>b</sup>          | 11963.16 <sup>c</sup>           | 11059.23 <sup>d</sup>           | P<0.01 |
| Average daily growth rate (g/day) | 0.29 $\pm$ 0.02 <sup>a</sup>  | 0.30 $\pm$ 0.04 <sup>a</sup>  | 3.48 $\pm$ 0.122 <sup>b</sup>   | 2.99 $\pm$ 0.45 <sup>c</sup>    | P<0.01 |
| Specific growth rate (%)          | 2.81 <sup>a</sup>             | 2.98 <sup>a</sup>             | 5.84 <sup>b</sup>               | 5.61 <sup>b</sup>               | P<0.01 |
| Condition factor                  | 0.714 <sup>a</sup>            | 0.650 <sup>b</sup>            | 0.837 <sup>c</sup>              | 1.260 <sup>d</sup>              | P<0.01 |

<sup>a,b,c,d</sup>Tukey HSD: Values on the same row with the same superscript are not significantly different ( $P>0.05$ ).

<sup>x,y</sup>Two samples t-test: Values on the same row with the same superscript are not significantly different ( $P>0.05$ ).



## FISHMEAL REPLACEMENT WITH OILSEED MEALS: EFFECT ON PHYSICAL CHARACTERISTICS OF PELLETED DIETS AND WASTE OUTPUT FROM NILE TILAPIA *Oreochromis niloticus*

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An 8-week trial was conducted to evaluate the effect of substituting fishmeal with cheaper and readily-available oilseed meal mixtures (soybean (SBM), cottonseed (CSM), groundnut (GNM) and copra (CM)) on some physical characteristics of the pelleted diets, faecal matter production as well as postprandial total ammonia nitrogen (TAN) and dissolved phosphorus (P) excretion rates of Nile tilapia. Six experimental diets (**D1-6**) with oilseed meals making up to ~80% of total dietary protein were formulated and a fishmeal-based diet as the control. The test diets had GNM, CSM, CM, and SBM in the following proportions: **D1** (5%, 15%, 25%, 35%), **D2** (15%, 25%, 35%, 5%), **D3** (25%, 35%, 5%, 15%), **D4** (35%, 5%, 15%, 25%), **D5** (20%, 20%, 20%, 20%) and **D6** (10%, 10%, 30%, 30%). Experimental unit used was a modified recirculation system stocked with 15 juvenile tilapia, with total bulk weight of ~1kg (mean weight of  $66.5 \pm 1.02$  g). TAN and P excretion rates were determined over a 24 hour period after three of the oilseed-based diets (1, 2 and 3) were administered at 2% of total fish weight in a single meal.

The dietary inclusions of the oilseed meal combinations resulted in increments in bulk densities between 46 and 63% (Figure 1), and faecal matter loads of 4 and 45% respectively, relative to the fishmeal control diet. The lowest and highest cumulative TAN excretion rates of  $106.14 \pm 1.95 \text{ mg kg}^{-1}$  and  $162.31 \pm 4.39 \text{ mg kg}^{-1}$  were recorded in fish groups fed the Diet 3 and control diet respectively (Figure 2). Accumulated P at the end of the 24-hour sampling period were  $127.15 \pm 4.16 \text{ mg kg}^{-1}$  for the control diet,  $45.11 \pm 7.78 \text{ mg kg}^{-1}$  for Diet 1,  $43.61 \pm 2.08 \text{ mg kg}^{-1}$  for Diet 2 and  $32.94 \pm 1.09 \text{ mg kg}^{-1}$  for Diet 3. Overall, the results of this study indicate the potential of minimising waste outputs using these oilseed mixtures as replacements to fishmeal in Nile tilapia diets, although, prior ingredient treatment such as solid-state fermentation might be necessary to reduce the high faecal loads.

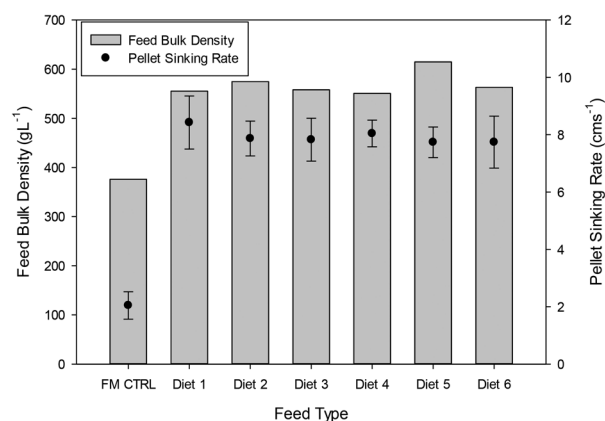


Figure 1. Pellet sinking rates and feed bulk densities ( $\pm$ SD) of the different experimental diets

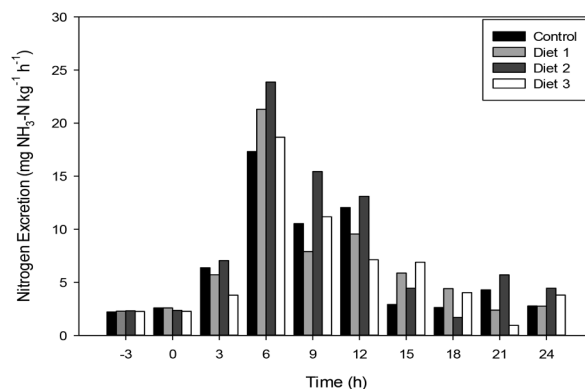


Figure 2. Postprandial total ammonia-nitrogen excretion rates (means and  $\pm$ SD) in *Oreochromis niloticus* fed three of the diets containing different plant protein matrices



## AN APPLICATION OF REMOTE SENSING FOR THE MONITORING OF SUSPENDED SEDIMENTS IN COASTAL AQUACULTURE

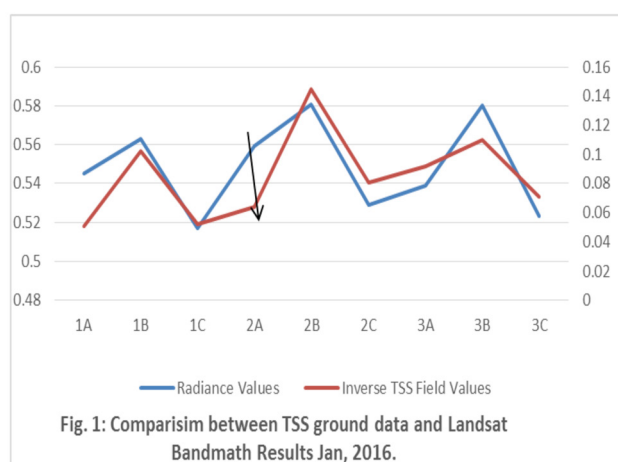
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As coastal farms rely on tidal movement for its water source, the disposal of wastes by coastal communities into River bodies may in most cases counteract processes of nutrient fluxes. Therefore an early detection of changing water color and increase in suspended solids through remote sensing will help prevent blooms and pollution of the water bodies as a result of waste disposal in neighboring coastal communities. This is essential to reduce causes of environmental changes and improve coastal aquaculture. One aspect of water quality analysis that has been proved through Remote sensing is water color, temperature and chl-a. However, more methods need to be applied for easy management of coastal water bodies and farms. This study was conducted to ascertain if band math equations using Landsat images will show a positive relationship with field in-situ data.

The study area for this research work is the Imo River estuary, Nigeria. There are a few brackish water farms which depend on the estuary for water. The sampling points chosen for this research were at N 04° 32.399' and E 007° 31.597', N 04° 30.979' and E 007° 32.455', N 04° 29.836' and E 007° 34.610' respectively. Water samples were collected from Kalibiam, Opobo and Queens Town, twice in the month of January 2016 to comply with the nearest available Landsat data acquisition on 4<sup>th</sup> of January 2016. The samples were analysed for Total suspended solids (TSS).

The Normalised Difference Suspended Sediment Index (NDSSI) was used. This model was developed for measuring suspended sediment levels in water bodies. Based on the transparency of water on the blue and infra red bands of Landsat images. The values derived was between +1 to -1. This study showed that the field results represented the inverse of the results obtained from the band math equation except in the station nearest to one of the largest community within the sampling area. The varying result in this sampling area can be associated to the difference in the day of Satellite image acquisition and field sampling.



## TRANSCRIPTOME ANALYSIS OF RAINBOW TROUT *Oncorhynchus mykiss* EGGS SUBJECTED TO THE HIGH HYDROSTATIC PRESSURE SHOCK

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The microtubule polymers of the mitotic spindle are sensitive to the hydrostatic pressure which applied to the fertilized fish eggs results in failure of the second body extrusion or/and inhibition of the first cell cleavage. Thus, the high hydrostatic pressure is applied in aquaculture to induce development of triploid sterile fish and diploidization of the gynogenetic and androgenetic haploid zygotes. It has been observed that high doses of the hydrostatic pressure applied to the fish eggs are also damaging for other cellular organelles and may affect early development of the fish embryos. The rainbow trout eggs contains maternal mRNA that is crucial for the proper development of embryos before the zygotic genome activation. So, it may be assumed that high hydrostatic pressure being damaging for the cell organelles may also affect cytoplasmic maternal transcriptome. Therefore, the main goal of the present research was to evaluate changes in the maternal transcriptome in eggs exposed for the high pressure shock.

Eggs from three females were collected in the separate plastic containers. Half of each batch of eggs were then exposed for 9000 psi of high pressure shock that lasted 3 minutes, a condition usually used for polyploidization of the genetic material in the rainbow trout eggs. Batches of the untreated eggs were kept at 4°C. RNA from both subjected to the high pressure shock and untreated eggs was extracted using modified TRIzol protocol. RNA quality was assessed and its integrity evaluated (Agilent's RIN) and compared between treated and untreated eggs. A total of 800 ng purified RNA was used as input for TruSeq RNA Sample Prep v2 kit (Illumina). Validated and normalized libraries were eventually sequenced using TruSeq SBSv3 Sequencing kit (Illumina) to obtain approx. 25 million reads per sample.

The mean RIN values for the treated and untreated eggs were similar. In total, 162.2 million sequencing reads were obtained and about 60% were successfully mapped to the reference transcriptome. 16,243 and 16,346 expressed transcripts were detected in the treated and untreated eggs, respectively. Differences in the expression level of transcripts between untreated eggs and eggs exposed for the high pressure shock were minor, however encompassed genes involved in the response to the DNA damage and repair, cell cycle control, chromatin remodeling and regulation, regulation of DNA synthesis and transcription (*IGF*, *RIF*, *CENPF*, *SETD2*, among others). Provided results indicated that the high pressure shock applied to the rainbow trout eggs to a certain extent effected maternal mRNA and may impair processes and mechanisms crucial for the proper development of the early fish embryos before activation of the zygotic genome.

This study was supported by the National Science Centre (NCN, Poland) project number 2014/15/BN/Z9/00510

## BOOSTING AQUACULTURE PRODUCTION IN AFRICA THROUGH STRATEGIC AND SYNERGISTIC PARTNERSHIPS AND ADVANCEMENT OF INTER/MULTI-DISCIPLINARY TRAINING

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Few universities in Africa offer specialised training in aquaculture at bachelors, masters or doctorate level; while those that do, have done so for relatively few years, not exceeding two decades. Traditionally, related aquaculture training in Africa was done under Zoology programmes, and was taught either as ichthyology, or fisheries biology, resulting into inadequacy in many skills and knowledge, and therefore necessitating “learning on the job” by the graduates. However, with increasing importance of the aquaculture sub-sector, as an alternative to dwindling catches from capture fisheries and its contribution towards provision of proteins and nutrition of many communities and demographic groups, there is now high demand for more specialised aquaculture training, to enable competent, professional and sustainable utilisation, improved production systems, and protection of water for production and other resources. Further, the advancement of knowledge and practices in aquaculture nowadays call for interdisciplinary and multidisciplinary training (Figure 1).

Although many of Africa’s practicing aquaculture scientists received their graduate training in Western Europe, Canada, North America, and Asia; during the past decade, there has been increasing effort geared towards establishment of programmes, facilities, infrastructures and funding, to provide training within African higher education institutions. To achieve specialised training in Africa, effective partnerships need to be established between collaborating institutions, to foster synergy in teaching, research and supervision, particularly at graduate level. Further, there is need to foster mobility of both students and staff, so as to harness limited resources and expertise, since no single African university currently has staff of all specialities. Indeed, innovations, such as, offering online courses and training; enabling credit transfer system among universities; joint supervision and examination of students; joint curriculum development and review would boost impactful training in Aquaculture.

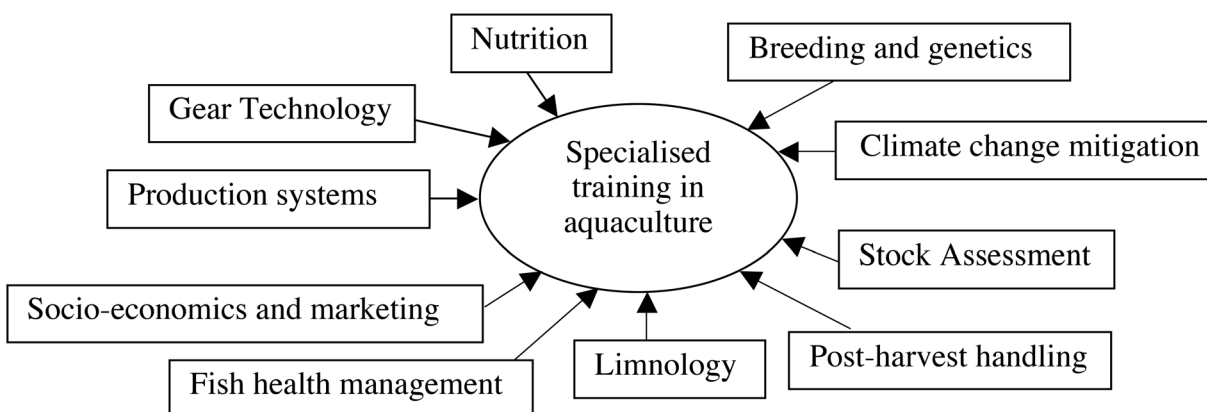


Figure 1. Interdisciplinary training required for effective aquaculture training in Africa

## SYNERGISTIC PARTNERSHIPS FOR INTER DISCIPLINARY TRAINING OF AQUACULTURE GRADUATES IN AFRICA

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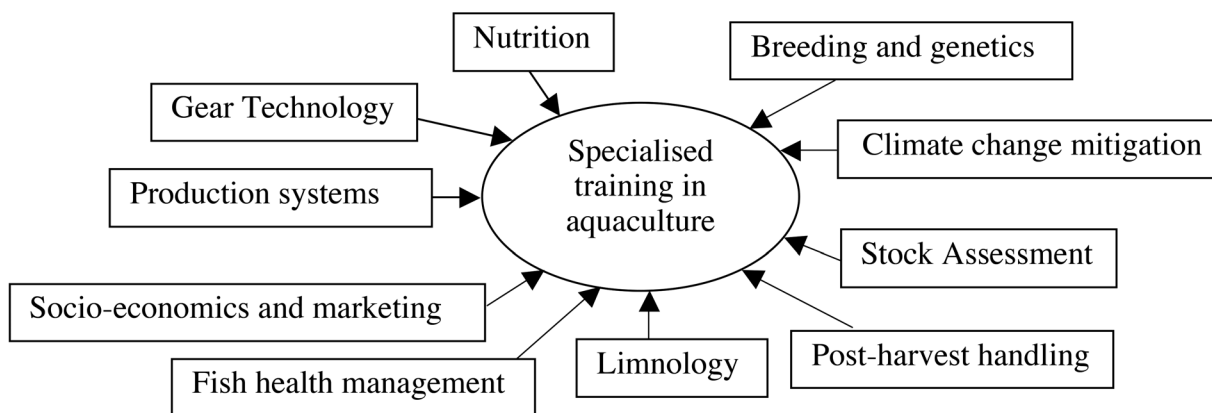


Figure 1. Interdisciplinary training required for effective aquaculture training in Africa

## ASSESSMENT OF CONSTRAINTS TO AQUACULTURE PRODUCTION IN NORTH CENTRAL, NIGERIA

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High cost of animal protein has forced people to rely on fish. In sum, Nigeria's fish import has been growing owing to the decline of her artisanal fisheries output. It is hoped that aquaculture will alleviate this problem. However, while aquaculture contribution to total fish is high in some developing countries, its contribution to total fish supply in Nigeria is far less than 10 percent, although it is growing at about 20% per annum in recent time. Serious developmental effort at aquaculture is still nascent. Hence, aquaculture is bedeviled by problems. This study assessed constraints to fish production in North Central, Nigeria using Kogi State as a case study. A three staged random sampling technique was used to select 200 cat fish farmers in the state. Data obtained through structured questionnaire were analyzed using mean score from a three point Likert type of scale. The findings indicated that the major constraint to cat fish production in the state is high cost of feed (M=2.80). This could be attributed to the fact that feed stuff and most inputs are sourced externally. Other problems include inadequate finance (M=2.61) and lack of encouragement from government (M=2.30). Amongst others, provision of inputs to cat fish farmers at subsidized rate was recommended.

**Table 1: Mean Score on Constraints to Aquaculture Production in North Central, Nigeria**

| Constraint                            | Mean | Rank             |
|---------------------------------------|------|------------------|
| Cost of feed                          | 2.80 | 1 <sup>st</sup>  |
| Inadequate finance                    | 2.61 | 2 <sup>nd</sup>  |
| Lack of encouragement from Government | 2.30 | 3 <sup>rd</sup>  |
| Predator                              | 2.20 | 4 <sup>th</sup>  |
| Inadequate electric power supply      | 2.02 | 5 <sup>th</sup>  |
| Inadequate research in aquaculture    | 1.86 | 6 <sup>th</sup>  |
| Lack of storage facility              | 1.89 | 7 <sup>th</sup>  |
| Poor harvesting tools                 | 1.61 | 8 <sup>th</sup>  |
| Cost of fingerlings                   | 1.36 | 9 <sup>th</sup>  |
| Inconvenience source of drug          | 1.34 | 10 <sup>th</sup> |
| Flood                                 | 1.11 | 11 <sup>th</sup> |
| Water pollution                       | 1.07 | 12 <sup>th</sup> |

## EFFECTS OF HYBRIDIZATION BETWEEN *Haliotis discus hannai* AND *Haliotis discus discus* ON ABALONE'S LONG-TERM GROWTH AND SURVIVAL

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Abalone is considered an important commercial species in the shellfish aquaculture industry. Though it accounts for only 3% of the total volume of the farmed shellfish, the species occupies over the half of the total value.

However, over-crowding and prolonged farming activities have caused problems like eutrophication, higher mortality and genetically inferior hatchery stock. As these problems are gradually lowering the productivity, many abalone farmers are looking for ways to shorten rearing period and to improve hybrid seeds with faster growth to overcome mortality.

To develop a hybrid abalone species with faster growth, this study conducted hybridizations between cold water *H. discus hannai* and warm water *H. discus discus* to produce both hybrid and pure hatchery stock and then compared growth and survival between the hybrid and pure seeds which were reared in indoor tanks and then seawater cages.

A 250 day long culture in indoor tanks showed the fastest growth in the hybrid *H. discus hannai* ♀ × *H. discus discus* ♂ with 33.9 mm shell length and 4.3 g weight, which was followed by the hybrid *H. discus hannai* ♀ × *H. discus hannai* ♂. After the 250 day long culture in tanks, seeds with about 34 mm shell length were selected from the tanks and transferred to seawater cages to be reared for another 800 days. The sea cage culture also showed the highest growth in the hybrid *H. discus hannai* ♀ × *H. discus discus* ♂ with 70.8 mm shell length and 45.8 g weight. Mortality of the pure *H. discus hannai* ♀ × *H. discus hannai* ♂, the hybrid *H. discus hannai* ♀ × *H. discus discus* ♂, the hybrid *H. discus discus* ♀ × *H. discus hannai* ♂, and the pure *H. discus discus* ♀ × *H. discus discus* ♂ during the 800 day cage culture was 18.9%, 16.6%, 19.4% and 17.8%, respectively, indicating the hybrid *H. discus hannai* ♀ × *H. discus discus* ♂ having the lowest mortality. Mortality mostly occurred in newly stocked small juveniles in the seawater cages. In conclusion, the comparison of growth and mortality between the hybrid and pure seeds reared in land-based tanks and seawater cages demonstrated that the hybrid *H. discus hannai* ♀ × *H. discus discus* ♂ performed the best.

## EFFECTS OF THE FEED ADDITIVE OF MARINE MICROORGANISM CONTAINING PIGMENT ON SHELL COLOR AND GROWTH OF *Haliotis discus hannai*

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Advancing farming technologies in the abalone aquaculture is increasing the use of formulated feed. Traditional feed of cultured diatom or seaweed is increasingly replaced with formulated feed containing animal protein for hatchery production. Despite its positive effect on short-term growth in abalone, the animal protein-based formulated feed is concerned to have negative effect on the physiological and health status of abalone in the long term.

To minimize the negative effect resulting from the use of formulated feed, we tried in this study to add marine microorganism containing pigment along with lactic acid bacteria into commercial feed products to enhance health and shell color of juvenile abalones. Juvenile abalones with an average shell length of 3.0 cm were stocked. 50 animals were stocked in each of the experiment tanks. The experiment groups include the feed groups added with astaxanthin 100, 1,000 and 3,000 ppm, the groups added with decaprenoxanthin 100, 1,000 and 3,000 ppm, the groups added with 10% and 50% lactic acid bacteria, and the control groups. The experiment groups were run in triplicate for 4 month rearing. Results from the 4 month rearing showed that the feed groups added with astaxanthin tended to increase weight gain, however all the other experiment groups added with decaprenoxanthin and lactic acid bacteria showed no effect on growth of shell length and weight of the stocked juveniles. It was also found that pigments derived from marine microorganism did not affect the color of the juvenile shell and muscle at all.



## GROWTH COMPARISONS OF HYBRID ABALONE SEEDS BETWEEN CHINESE AND KOREAN POPULATIONS OF *Haliotis discus discus*

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The abalone aquaculture industry in Korea is mainly based on the cold water abalone, *H. discus hannai*, on which breeding research and development is actively carried out for genetic improvement of hatchery stock. Meanwhile, the global climate change is steadily warming the water temperature in Korea's coastal areas in summer, causing higher mortality in abalone farms. To counter this problem, efforts are being made to domesticate the warm water species, *H. discus discus* for aquaculture or to develop hybrid hatchery stock between warm water *H. discus discus* and cold water *H. discus hannai* to obtain higher growth and temperature tolerance to warmer water. In addition, to prevent the potential inferiority in hatchery stock due to prolonged farming activities in limited areas, efforts are also being made to improve genetic diversity of hatchery stock through hybridization between a Chinese population and a Korean population of *H. discus discus*.

This study conducted comparison of growth of hybrid seeds between the *H. discus discus* population introduced from Qingdao, China and the Korean population at three different temperature conditions. Hybrid seeds from Chinese *H. discus discus* ♀ × Chinese *H. discus discus* ♂, Chinese *H. discus discus* ♀ × Korean *H. discus discus* ♂, Korean *H. discus discus* ♀ × Chinese *H. discus discus* ♂, Korean *H. discus discus* ♀ × Korean *H. discus discus* ♂ were simultaneously produced to be used in the experiments. At the start of the experiments, the average shell length and weight was 43.5 mm and 8.8±0.3 g, respectively and the experiments were conducted in triplicate at 3 different temperature conditions (15, 20, 25°C). At day 200 of the experiment under the condition of 25°C, hybrid seeds from Korean *H. discus discus* ♀ × *H. discus discus* ♂ performed best exhibiting 54.2 mm in shell length, 18.6±2.0 g in weight. It can be concluded that the pure seeds from the Korean population grow faster than the hybrid seeds between the Chinese and Korean populations. It may be explained by the fact that the place of origin of the Chinese population, Qingdao has relatively lower water temperature than that of the Korean population, Jeju and thus maybe less tolerant to warmer water.

## THE EFFECTS OF POLLUTION ON FISH HEALTH AND THE MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES IN FISH

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Pollution is the introduction of contaminants into a natural environment which leads to instability, disorder, harm or discomfort to, either physical systems or living organisms in the ecosystem. Potentially harmful substances such as pesticides, heavy metals, paper mill waste, polychlorinated biphenyl and crude oil are often released into the aquatic environment. When large quantities of these pollutants are released there may be an acute impact as measured by large-scale sudden mortalities of aquatic organisms such as fish kills. Lower levels of discharge may result in an accumulation of the pollutants in body composition of fish. The effects of water pollution on fish species can be classified into acute effects and long term chronic effects, which includes immune-suppression, reduced metabolism, and damage to gills and epithelia. With increasing industries, agricultural activities, urbanisation and tourism every year, anthropogenic activities release several tonnes of chemicals into the water. Pollution-related diseases include fin/tail rot, gill disease, hepatic damage and ulceration. Specific examples of fish diseases that reflect the effects of pollution include, fin and tail rot caused by *Ammonas hydrophila* and *Pseudomonas jluorescens*. The polluted water may have undesirable colour, odour, taste, turbidity, harmful chemical contents, toxic and heavy metals, pesticides, industrial waste products, domestic sewage, virus, bacteria, protozoa and worms. Tilapia species are not only tolerant to severe contaminated waters but show a variety of specific morphological deformities and lesions that appear to reflect the level of water pollution. Assessing morphological deformities is one of the most straightforward methods to study the effects of contamination on fish because of the ease of recognition and examination when compared with other types of biomarkers. The indiscriminate disposal of pollutants without pre-treatment should be discouraged. In order to avoid the effects of water pollution on fish health, effluents discharged from industries and other sources should be properly treated.

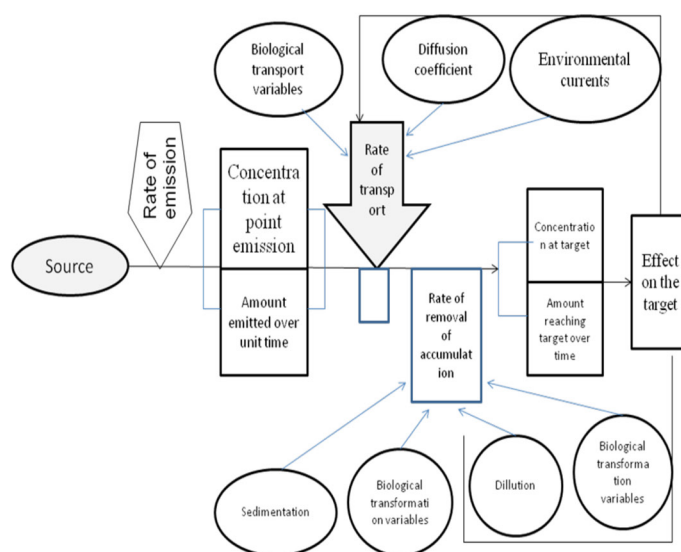


Figure 1: A generalized pollutant pathway

Source: Holdgate, (1979)

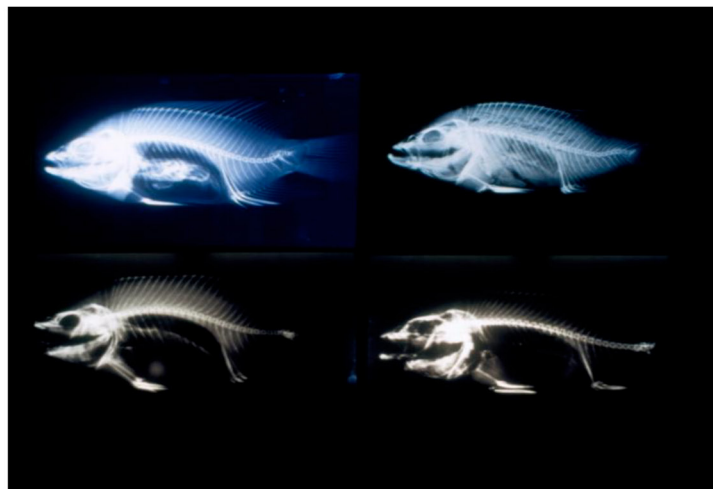


Plate 1: Different Types of Skeletal Deformities in Tilapia

Source: Brown-Peterson, (1998)

## AFLATOXIN LEVELS IN SELECTED FISH FEEDSTUFFS AND FISH FEEDS SOLD IN LAGOS, NIGERIA

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Mycotoxins are secondary metabolites produced by fungi, commonly referred to as molds. Aflatoxins are mycotoxins produced by *Aspergillus flavus* and *A. parasiticus* group of fungi.

Aflatoxins and other mycotoxins are frequently detected in feedstuffs, commercial fish-feeds and some aquatic fauna. The ingestion of aflatoxin contaminated diets can lead to hazardous effects on fish production and health.

This study determined the level of aflatoxin contamination in some fish feeds and fish feed ingredients sold in Lagos, Nigeria. Sixty two (62) samples: fish feed (12), fishmeal (10), groundnut cake (10), soybeans meal (10), wheat offal (10) and crushed maize (10) were randomly purchased from an agro feeds market in Lagos. Aflatoxin analysis was carried out using the Enzyme-Linked Immunosorbent Assay (ELISA) method- RIDASCREEN FAST Aflatoxin Total.

Aflatoxin contamination was detected in approximately 50% of the fish feeds, while 70 – 100% of the feedstuffs were highly contaminated. The aflatoxin concentrations in the samples ranged from 1.85 - > 45 µg/Kg. However, there were no significant differences in the concentration of aflatoxin in the fish feeds, fishmeal, soybeans meal and wheat offal, but these concentrations were significantly lower than those of groundnut cake and crushed maize. The moisture content of the samples was not significantly different ranging from 1.86 to 2.44%. Most of the feedstuffs (especially maize and groundnut cake) were highly contaminated.

There is therefore a need to create more awareness among fish farmers, agro feedstuff traders; agro feed processors and the general populace on the need to source for good quality feedstuffs and the inherent dangers in the use of aflatoxin-contaminated fish feeds and feedstuffs.

## PERFORMANCE AND HISTOLOGICAL ALTERATION OF AFRICAN CAT FISH *Clarias gariepinus* FED YELLOW CORN WASTE MEAL

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The study was conducted to assess the potential use of yellow corn waste meal as a substitute energy source and to investigate its histopathological effect on the gills and the kidney of African cat fish. A total of One hundred and eighty African cat fish fingerlings (average weight 6.88kg+/-) divided into six groups of treatments were fed 3% body weight of yellow corn waste based diets at varying levels (0%, 10%, 20%, 30%, 40% and 50% respectively) containing 40% crude protein for a period of 70 days. All the growth parameters determined were significantly different ( $p < 0.05$ ). Highest MWG, PWG and SGR (13.290g, 192.33% and 1.54 %/day) were observed in treatment 4 and the least value of 5.470g, 76.05% and 0.84 %/day were recorded in treatment 6 respectively. Highest AFI 23.02g was recorded in treatment 3 while the least value of 15.37g was recorded in treatment 5. However, highest FCR of 3.10 was recorded in treatment 6 while treatment 4 had the least value of 1.59. Treatment 3 and 4 had the highest PI and PER (9.20 and 1.57) while the least value of 6.15 and 0.01 were recorded in treatments 5 and 6 respectively. The gills tissues of the controlled fish, Plate A showed hyperplasia of the secondary lamellae, mucous cells and Central venous sinus. Treatment 2, Plate B showed mild fusion of secondary lamellae, the third treatment plate C demonstrated lot of cellular necrosis, hyperplasia of secondary lamellae and primary lamellae and distortion of central venous sinus. The fourth treatment Plate D demonstrate lot of cellular necrosis and hyperplasia of secondary lamellae and primary lamellae and distortion of central venous sinus. The fifth treatment, had degeneration in the secondary lamellae and epithelial cells, it also shows hyperplasia of the central venous sinus. The gill tissue in the treatment six (plate F) demonstrated degenerated tissue. 10%, 20%, 30%, 40% showed histopathological alterations in their kidney such as Total rupture of the cells, Necrosis, hypertrophy of the glomerulus, degradation of; the renal tubules, Bowman's capsule and corporsule and shrinkage of the cells. In the experiment, normal kidney histology was observed only at 50% inclusion. In conclusion, yellow waste meal can replace yellow maize up to 40% without any adverse effect on the gills and kidney of African catfish *Clarias gariepinus*.

**TABLE 1 GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF *Clarias gariepinus* FINGERLINGS FED VARIOUS LEVELS OF YELLOW CORN WASTE MEAL.**

| PARAMETERS   | Treatments          |                     |                     |                    |                     |                    | SEM   |
|--------------|---------------------|---------------------|---------------------|--------------------|---------------------|--------------------|-------|
|              | 1                   | 2                   | 3                   | 4                  | 5                   | 6                  |       |
| IWG (g)      | 6.88                | 6.89                | 6.87                | 6.91               | 6.88                | 6.92               | 0.015 |
| FWG (g)      | 13.88 <sup>d</sup>  | 18.15 <sup>c</sup>  | 19.80 <sup>b</sup>  | 20.20 <sup>a</sup> | 12.40 <sup>e</sup>  | 12.39 <sup>e</sup> | 0.811 |
| MWG (g)      | 7.00 <sup>d</sup>   | 11.26 <sup>c</sup>  | 12.93               | 13.29 <sup>a</sup> | 5.52 <sup>e</sup>   | 5.47 <sup>e</sup>  | 0.811 |
| ADWG (g/day) | 0.10 <sup>a</sup>   | 0.16 <sup>a</sup>   | 0.18 <sup>a</sup>   | 0.19 <sup>a</sup>  | 0.08 <sup>a</sup>   | 0.08 <sup>a</sup>  | 0.019 |
| PMWG (%)     | 101.7 <sup>4c</sup> | 163.4 <sup>3d</sup> | 188.2 <sup>1b</sup> | 192.               | 179.6 <sup>5c</sup> | 76.05 <sup>1</sup> | 10.89 |
| SGR(%/day)   | 1.00 <sup>c</sup>   | 1.39 <sup>b</sup>   | 1.51 <sup>ab</sup>  | 1.54 <sup>a</sup>  | 0.84 <sup>d</sup>   | 0.84 <sup>d</sup>  | 0.075 |
| AFI(g)       | 15.88 <sup>c</sup>  | 19.40 <sup>c</sup>  | 23.02 <sup>a</sup>  | 21.10 <sup>b</sup> | 15.37 <sup>1</sup>  | 16.95 <sup>d</sup> | 0.679 |
| FCR          | 2.27 <sup>c</sup>   | 1.72 <sup>c</sup>   | 1.78 <sup>d</sup>   | 1.59 <sup>c</sup>  | 2.80 <sup>b</sup>   | 3.10 <sup>a</sup>  | 0.139 |
| PI (%)       | 6.35 <sup>c</sup>   | 7.76 <sup>c</sup>   | 9.20 <sup>a</sup>   | 8.44 <sup>b</sup>  | 6.15 <sup>1</sup>   | 6.78 <sup>d</sup>  | 0.271 |
| PER(g)       | 1.10 <sup>c</sup>   | 1.45 <sup>ab</sup>  | 1.41 <sup>b</sup>   | 1.57 <sup>a</sup>  | 0.89 <sup>d</sup>   | 0.01 <sup>c</sup>  | 0.129 |

a, b, c and d Mean within the same row with different superscripts differ significantly  $p < 0.05$

## CAN FISH FARMING TRANSFORM FOOD SECURITY IN RURAL KENYA?

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Though the bulk of current aquaculture production occurs in China, certain sub-Saharan African countries are making increasingly meaningful forays into the sector. Many of these interventions are born out of the hope that fish farming – when directed toward rural communities – can bring about transformative food security gains. This research, conducted as part of a PhD dissertation at Stanford University, contributes to the growing body of scientific literature from the continent that takes on the question: to what extent, and under what conditions can small-scale fish farming improve food security (measured in terms of access, availability and utilization)? Research occurs in Kenya, and focuses on a government-led fish farming intervention that ran from 2009 – 2012. By collecting and analysing socioeconomic survey data from 1000+ fish farming households, this research is able to get at the ‘pro-poor’ dimensions of the intervention.

Results indicate positive nutritional and income gains for adopting households, but also show that well diversified ‘middle-income’ households tend to benefit the most. Indeed, high rates of pond abandonment amongst poorer households paint a concerning picture. The results also indicate that while farmers utilizing ‘improved’ on-farm practices enjoy higher yields, underdeveloped input and output markets significantly impact their profits (Table 1). Recommendations include (1) orienting future programming towards smaller subsets of farmers who are most likely to succeed (and then act as “early adopters” in their communities), and (2) fostering private sector growth across the value chain, primarily in order to bring down the cost of inputs.

| <b>Table 1. The effects of on-farm practices on yield and profit</b>  |                    |            |                             |              |                        |                               |              |                        |
|---|--------------------|------------|-----------------------------|--------------|------------------------|-------------------------------|--------------|------------------------|
| <b>T-tests compare pond level yields and profits for a variety of on-farm management practices and input choices.</b> |                    |            |                             |              |                        |                               |              |                        |
|   |                    |            | <b>Yield (kg/pond/year)</b> |              |                        | <b>Profit (USD/pond/year)</b> |              |                        |
|   |                    | <b>N</b>   | <b>Mean</b>                 | <b>Error</b> | <b>Mean difference</b> | <b>Mean</b>                   | <b>Error</b> | <b>Mean difference</b> |
| <b>Fertilizer</b>   | <b>Yes</b>         | <b>783</b> | <b>309 kg</b>               | <b>10.1</b>  | <b>0.002***</b>        | <b>\$318</b>                  | <b>17.8</b>  | <b>0.007***</b>        |
|   | <b>No</b>          | <b>61</b>  | <b>207 kg</b>               | <b>30.2</b>  |                        | <b>\$162</b>                  | <b>52.9</b>  |                        |
| <b>Fingerling Sex</b>   | <b>Monosex</b>     | <b>360</b> | <b>343 kg</b>               | <b>16.8</b>  | <b>0.001***</b>        | <b>\$342</b>                  | <b>24.9</b>  | <b>0.20</b>            |
|   | <b>Mixed sex</b>   | <b>395</b> | <b>275 kg</b>               | <b>12.4</b>  |                        | <b>\$296</b>                  | <b>26.8</b>  |                        |
| <b>Fingerling Source</b>  | <b>Purchased</b>   | <b>673</b> | <b>312 kg</b>               | <b>11.3</b>  | <b>0.06*</b>           | <b>\$306</b>                  | <b>19.7</b>  | <b>0.54</b>            |
|   | <b>Self-bred</b>   | <b>134</b> | <b>265 kg</b>               | <b>21.5</b>  |                        | <b>\$331</b>                  | <b>36.8</b>  |                        |
| <b>Culture</b>  | <b>Monoculture</b> | <b>636</b> | <b>308 kg</b>               | <b>10.9</b>  | <b>0.44</b>            | <b>\$309</b>                  | <b>19.8</b>  | <b>0.56</b>            |
|   | <b>Polyculture</b> | <b>137</b> | <b>286 kg</b>               | <b>26.4</b>  |                        | <b>\$281</b>                  | <b>44.9</b>  |                        |
| <b>Feed</b>   | <b>Purchased</b>   | <b>285</b> | <b>321 kg</b>               | <b>17.4</b>  | <b>0.12</b>            | <b>\$320</b>                  | <b>31.0</b>  | <b>0.51</b>            |
|   | <b>Homemade</b>    | <b>127</b> | <b>276 kg</b>               | <b>23.4</b>  |                        | <b>\$284</b>                  | <b>45.6</b>  |                        |

\*p<0.10

\*\*p<0.05

\*\*\*p<0.01

## GAPS IN THE TECHNOLOGY DEVELOPMENT FOR THE CULTURE OF SIX NATIVE BURROWING BIVALVES OF COMMERCIAL INTEREST IN CHILE

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The increase in the exploitation of clams has produced a reduction in the artisanal CPUE and landing. Therefore, the importance of developing a technology for culture and commercial production of burrowing bivalves is evident. The aim of this work is to make an analysis of bivalves with biologic and market potential in Chile, to establish the state of the art and detect the gaps to complete and improve the current cultivation technology.

According to the results of an advisory contracted by the government and an expert team, we include six species in this analysis: *Mulinia edulis* (taquilla clam), *Venus antiqua* (taca clam), *Ensis macha* (razor clam), *Gari solida* (huepo), *Mesodesma donacium* (surf clam) and *Tawera gayi* (juliana clam) (Table 1).

Presently, there are no commercial cultures of clam in Chile, nevertheless two species are more advanced, *M.edulis* and *V.antiqua*. The effect of culture density, diet and ration in the growth and survival of larvae and post larvae of *Mulinia* (Oliva *et al.* 2013, 2014; Vivanco *et al.*, 2014) is known. Grown out experiments in the sea showed that seeds produced in hatchery are capable of surviving long transfer periods and grow both in the subtidal and intertidal zone (Abarca *et al.*, 2012). In the case of *Venus antiqua*, several studies have been made on reproductive cycle, broodstock conditioning, larvae and postlarvae culture, seed production (Bustos and Olavarria, 2000) and growing up in the sea up to commercial size.

In razor and surf clam, larval and post-larval cultures are developed, with a pilot level scaling up to production of competent larvae in *Ensis* (Arriagada *et al.*, 2013).

*Gari solida* currently has initial research on embrionary development and culture of larvae and post larvae (Olavarria *et al.*, 1996, Contreras *et al.*, 2014 a, b). The scaling of the larval and postlarval cultures of this species is still lacking.

The culture of *Tawera gayi* is already starting in an initial experimental level.

| Stage/Species          |                      | <i>Mulinia edulis</i>         | <i>Venus antiqua</i>          | <i>Ensis macha</i>       | <i>Mesodesma donacium</i>     | <i>Gari solida</i>            | <i>Tawera gayi</i>            |
|------------------------|----------------------|-------------------------------|-------------------------------|--------------------------|-------------------------------|-------------------------------|-------------------------------|
| Hatchery and Nursery   | Larval culture       | Technology developed          | Technology developed          | Technology developed     | Pilot level technology        | Experimental level technology | Experimental level technology |
|                        | Post-Larvae culture  | Technology developed          | Technology developed          | Pilot level technology   | Experimental level technology | Experimental level technology | Technology not developed      |
| Grown-out in the field | In suspended systems | Pilot level technology        | Pilot level technology        | Technology not developed | Technology not developed      | Technology not developed      | Technology not developed      |
|                        | In bottom systems    | Experimental level technology | Experimental level technology | Technology not developed | Technology not developed      | Technology not developed      | Technology not developed      |

■ Technology developed   
 ■ Pilot level technology   
 ■ Experimental level technology   
 ■ Technology not developed

Figure 1: Hatchery, nursery and grown-out knowledge in six native burrowing bivalves

## **VULNERABILITY, RISK MANAGEMENT AND COPING STRATEGIES OF CLIMATE VARIABILITY AMONG THE FISH FARMERS IN ABUJA AREA COUNCILS**

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Aquaculture sector in the last two decades has recorded rapid growth among the food producing sectors and has developed into a globally robust and vital industry. Its contribution to global per caput fish production has grown from 0.7kg in 1970 to 8.4kg in 2010 and it is still growing in many rural, urban and peri urban areas with few employment opportunities, aquaculture is fast becoming the main drivers of local economies with fish as an important source of food security worldwide. However, this important sector is under serious threat as production is on the downward trend with the complications arising from the negative impact of variability in climate which results in the variation in temperature, irregular rainfall pattern, flooding, low rainfall pattern and increased incidence of diseases. The study thus looks at Vulnerability risk management and coping strategies of climate variability impart among the fish farmers in Abuja area councils FCT

Simple random sampling technique was employed in selecting the respondents (the fish farmers). Seventy structured questionnaires were administered in order to get the information needed on the climate risks and vulnerability on fish farmers. Data collected include the socioeconomic parameters of the fish farmers, livelihood and production data. Data were analysed using descriptive statistics (mean, frequency standard deviation) and Multinomial Logistic Regression analysis.  $\alpha = 0.05$

The result shows 70% of the respondents were male while 30 % were female, 96.3% of the respondents fell between ages 23 and 62 years, 79.4% had one form of education or the other. About 67.1% had household size of between 2– 7 people. Unpredictable weather condition resulting in flooding, drought and strong wind were the environmental threats with the highest occurrence while increased fish diseases, low hatchery resulting in shortage of fish seed . Support from family members, construction of dykes and change in management strategies were factors that had positive significance on productivity for both male and female while livelihood diversification had a negative significance on the productivity of the male fish farmers

Strengthening aquaculture management practices with adequate water utilization and conservation should be encouraged. Pond construction and fish seed production should be given priority. Climate smart technology should be employed to minimize the risk.



## QUALITY, SAFETY AND INTERNATIONAL TRADE OF AQUACULTURE PRODUCTS IN OYO STATE NIGERIA

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Research was conducted on the quality, safety and trade of aquaculture products in Ibadan metropolis. Production of farmed fish is on the increase but most are still consumed locally, thus limiting production level. It was found that some factors affect the quality, safety and trade of fish products in the metropolis. The study sought to identify the constraining factors in the marketing and trade of aquaculture products, the need for improving the quality, its safety to the health of the consumers, the rationale of what has led to the decline in the productivity levels and the possibility of trading internationally so as to improve the livelihood of the farmers

A purposive sampling technique has been used to select 58 respondents from Ibadan metropolis. Structured questionnaire and interview were used to collect data on respondents' demographic characteristics such as their gender, household sizes, occupation ethnicity, quality and safety aspects.

The study conducted was analysed using descriptive statistics, (frequency tables) logistic regression –Probit and difference of means.

The result shows that percentage gender of male to female were 60.3% and 39.7%. The most ethnicity amongst respondents are Yoruba (82.8%). Most of the results had attained tertiary level of education (89.7%). The result of the analysis at 10% level of significance shows that foul smell, mould, and presence of maggot were the major quality concern at the point of sale. Analysis also shows the likelihood of the farmer to trade locally or internationally. Inability to obtain the sufficient loan, less quantity produced and the degree of measure of quality of the products shows that the farmer might result to local trade only.

To be able to meet international standard and compete effectively globally, there is the need to sustain regular production through credit access and enhance the quality of final product.

## **DETERMINANTS OF OUTPUT OF SMALL SCALE FISH FARMERS IN IKORODU LOCAL GOVERNMENT AREA, LAGOS STATE**

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Among Nigerians there have been problems of low protein intake which has necessitated importation of stock fish, frozen fish, meat etc. to forestall an impending food crisis (Osugiri et al, 2007). This study therefore sets out to analyze the determinants of output of small-scale fish farm operators in Ikorodu Local Government Area of Lagos State. Identification of factors that determines output of fish farmers will help policy makers to design intervention programme that will enhance the output of fish farm operators in order to realize one of the sustainable development goal to reduce hunger.

Purposive simple random sampling technique was used in the selection of respondents. Ikorodu local government was purposively selected because of the presence of fish farming estate hosting 250 fish farmers which serves as the sampling frame. From the 250 fish farmers, 101 fish farm operators were randomly selected. Data were obtained from the respondents socioeconomic characteristics, type and quantity of fish stocked, quantity harvested and sold, cost of production, profit realized among others. The data obtained were analyzed using Descriptive statistics, Budgetary analysis, Gini Coefficient and Ordinary least square regression.

Majority of those involved in fish farming are male (65.3%) married (54.5%) and have tertiary education (69.3%). The mean age, household size, pond size, years of farming experience are 36.44, 4.03, 39.78 and 2.67 respectively. The result shows that there is wide variation in the output of fish farm operators with about 88.5% disparity in their output. The mean profit made per fish sold in the study area is ₦186.24. Factors that positively determine the output of fish farmers are years of experience in fish farming (p-value 0.001), volume of credit obtained (p-value 0.001) and quantity of feed used (p-value 0.001) while there is a negative but significant relationship between age of household head and the output of fish farmers (p-value 0.005).

The study therefore shows that fish farming is a profitable business with varying degree of output. In order to improve the output of fish farm operators, government and relevant stakeholders in fisheries management should ensure that fish farmers do not only have access to credit facilities but in required amount. In addition, feed is an important determinant of output of fishing, therefore there is the need for quality and adequate feeding of fish by fish farmers which should be made available and affordable to them.

## **CHEMICAL AND NUTRITIONAL VALUE OF MELON SHELL AS POSSIBLE FISH FEED INGREDIENT**

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A study to evaluate the chemical and nutritional value of melon shell of fish feed ingredient was carried out. Samples of melon shell were collected from yenagoa market and taken to the laboratory for proximate analyses. Result shows moisture content value was 8.9%, crude protein 10.2%, crude fiber 4.5%, Ether extract 16.03%, while the ash content 6.23%. Crude protein value of melon shell shows no difference when compared with maize value 10.0%. This study shows that melon shell can conveniently replace maize in the formulation of fish feed.

## **EFFECT OF DIETARY TREATMENT ON THE HEMATOLOGY OF CATFISH (*Clarias gariepinus*) FINGERLINGS**

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A study to evaluate the effect of graded level substituting maize with melon shell on hematology of *Clarias gariepinus* fingerling was carried out. Five diets were prepared to have 40% CP. Melon shell were used to replace maize at the rate of 0% (diet one), 25% (diet two), 50% (diet three), 75% (diet four), and 100% (diet five). Each treatment had three replicates using 30 *Clarias gariepinus* fingerlings per replicate. The fingerlings were fed at 3% body weight per day for 12 weeks. Result obtained showed blood parameters (PCV, HB, RBC, WBC, and Plasma protein) were significantly different ( $P < 0.05$ ) among experimental fishes. Higher level of PCV, WBC were observed in fish fed diet five respect to other diets while Hb, RBC and plasma protein were higher in fishes fed diet four. Our finding shows significant change in hematology values of experimental fish increase as inclusion level of melon shell increase in the diets. The differences found in this study could be attributed to the inclusion of melon shell in the diets.

## PRODUCTIVITY AND PROFITABILITY OF AQUACULTURE ENTERPRISES IN UGANDA SOUTH EASTERN AGRO-ECOLOGICAL ZONE

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Enterprise analysis of aquaculture in Uganda is necessary to assess the economic viability of the sector and provide guiding information to investment. This analysis was performed to establish the productivity and profitability of aquaculture production in south eastern agro-ecological zone of Uganda (U'SEA EZ). Data were obtained from a census database of Buginyanya ZARDI that was earlier generated through enumeration of aquaculture establishments, using hand delivered questionnaire, on-site and laboratory analysis of water quality parameters of the existing systems. Production, species cultured and related factors were analyzed using one-way analysis of variance (ANOVA), Chi-square test and regression models using SPSS and STATA versions 17 and 14 respectively. Mean productivity ( $\chi^2 = 1.2223$ ;  $df = 6$ ;  $p = 0.976$ ) ranged from 0.301 to 2 kgm<sup>-3</sup>, mainly in earthen ponds. Production decreased from over 500mt in 2010 to about 100mt in 2014 and increased over 10 fold in 2015. Nile tilapia, African catfish were the most cultured fish species while the Mirror carp was cultured in lower scale by only 5% of the farmers. Unit fixed cost was higher (11,175.71/= about \$ 3.19) in the 0.301 kgm<sup>-3</sup>, than in 2 kgm<sup>-3</sup>, that was 6,141.67/= (\$1.75) while variable cost per kg fish was higher (5,940; \$1, 70) at carrying capacity of 2kgm<sup>-3</sup> than at 0.301kgm<sup>-3</sup> which was 5,323.59 (\$1.52). Total cost per kilogram was higher (54814.95; \$15.66) at 0.301kgm<sup>-3</sup> than 6,041 (\$1.73) in the 2kgm<sup>-3</sup> respectively. Production was profitable at 2 kgm<sup>-3</sup> (1,959.20/= (\$0.52kg<sup>-1</sup>). Production and profitability were mainly influenced by farmers' knowledge of fish nutrition ( $\chi^2 = 46.3534$ ;  $P = \leq 0.001$ ), site suitability  $F(=2, 82) = 8.12$ ;  $P = 0.001$ , water quality and carrying capacity of the systems. Overall, aquaculture enterprise in U'SEA EZ is a profitable venture at carrying capacity  $\geq 2\text{kgm}^{-3}$ , yielding ROI of 32%.

## COST EFFICIENCIES OF CATFISH FIRMS' IN KOGI STATE, NORTH CENTRAL NIGERIA

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In this Study focused, cost efficiency levels of catfish producing firms of Kogi State, north central Nigeria were estimated. Stochastic frontier cost function was applied to data obtained from 100 small scale catfish farmers. These catfish farmers were selected through a multistage sampling procedure. Results reveal that the venture is dominated by male. These fish farmers are aging, highly educated but have an average experience of 6 years. Costs of land and labour were significant determinants of cost inefficiency. Less than half (42 farmers or 42% of the Farmers) incur 5% more than the economically optimum cost. These constitute the most cost inefficient firms. Thus, allocative efficiency in catfish production in the area is fair. The average cost efficiency level in the area is 1.106, suggesting that farmers on the average incur about 11% more cost than necessary. The level of cost efficiency could improve with attempts at reducing inefficiency by this 11%. To achieve this, catfish farmers must pay some attention to pricing of land and labour, and seek to optimize the use of these factors of production. This can be achieved through training on cost and input relationship.

**Table 1: Stochastic cost frontier estimates for catfish production in Kogi state**

| Variables        | Parameter  | Coefficient | t-ratio     |
|------------------|------------|-------------|-------------|
| Constant         | $\beta_0$  | 76725.893   | 79193.568*  |
| Output           | $\beta_1$  | 0.924       | 3.334*      |
| Feed Cost        | $\beta_2$  | 0.084       | 0.298       |
| Pond Cost        | $\beta_3$  | 0.057       | 0.576       |
| Land Cost        | $\beta_4$  | -25610.850  | -46619.614* |
| Labour Cost      | $\beta_5$  | 25611.025   | 31179.520*  |
| Treatment        | $\beta_6$  | 0.042       | 0.450       |
| Cost/drugs       |            |             |             |
| Fingerlings Cost | $\beta_7$  | 0.630       | 1.058       |
| Sigma square     | $\delta^2$ | 0.673       | 1.8**       |
| Gamma            | $\Gamma$   | 0.968       | 3.696*      |

\*, \*\* = Significant at 1%, 5%

**Table 2: Cost efficiency estimates of catfish firms in Kogi state**

| Efficiency Range | Frequency | Percentage |             |
|------------------|-----------|------------|-------------|
| 1.00-1.05        | 58        | 58.00      |             |
| 1.05-1.10        | 29        | 29.00      |             |
| 1.11-1.15        | 4         | 4.00       |             |
| 1.15 +           | 9         | 9.00       |             |
| Total            | 100       | 100.00     | Mean: 1.106 |

## EFFECT OF STEAMING DURATION OF *Moringa olefera* LEAF ON THE GROWTH AND NUTRIENT UTILIZATION ON *Clarias gariepinus* FINGERLINGS

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Aquaculture has been globally recognized as the fastest growing sector of the world's animal production contributing more than 19million metric tons of fish and shellfish yearly to the world's fish. To sustain such high rates of increase in production, a matching increase in fish feed production is imperative. Fish feed accounts for between 60 – 70% of the variable cost of cost in fish culture, due to the high cost of feed ingredients which has made researchers to look inwards for cheaper sources of energy. Leaf meal proteins are among the unconventional sources of protein that may reduce the high cost of fish feed. A particular leaf meal of interest as a potential dietary protein is *Moringa olefera*. Hence a feeding trial which lasted for 10-weeks was conducted to compare the growth performance of *Clarias gariepinus* fingerlings having an average weight of 6.65±0.20 fed *Moringa* leaves steamed at different duration of 15 minutes (diet 2), 30 minutes (diet 3), 45 minutes (diet 4) and 60 minutes (diet 5), while the control (diet 1) contained no *moringa* acted as the control. The fish was stocked in triplicate group per treatment.

The mean weight gain (MWG) of the fish fed diet 1,2, 3, 4 and 5 were 8.95g, 8.95g, 14.41g, 11.37g and 15.70g respectively. The fingerlings fed diet 5 had the highest percentage weight gain (PWG) value (235.74%) while those fed diet 1 had the lowest value (134.64%). In the same vein, the highest specific growth rate (SGR) of 0.74% was observed in fingerlings fed diet 5. Those fed with Diet 1 and 2 had the least value of 0.53. The feed conversion ration (FCR) observed in fingerlings fed diet 1 (4.28) was higher than those fed other diet, with the lowest value in fingerlings fed diet 3 (3.24). Fingerlings fed diet 1, 3 and 4 had similar Protein Efficiency ration of 0.49 while fingerlings fed diet 2 and 5 also had the same result of 0.47. Based on these result, fish fed with *moringa* leaves steamed for duration of the 60 minutes (Diet 5) performed better without compromising growth rate or causing any deleterious effect on the fish. It is hence concluded that *moringa* leaves steamed for duration of 60 minutes can be included in the diet of *Clarias gariepinus* fingerlings.

Table 1: Growth performance and Nutrient Utilization of *Clarias gariepinus* Fed Experimental Diets.

| Parameters                  | 1      | 2      | 3      | 4      | 5      | Mean   | SD   | S.E±  |
|-----------------------------|--------|--------|--------|--------|--------|--------|------|-------|
| Initial mean weight (g)     | 6.6    | 6.52   | 6.23   | 6.52   | 6.66   | 6.51   | 0.08 | 0.08  |
| Final mean weight (g)       | 15.58  | 15.47  | 20.64  | 17.89  | 22.36  | 18.39  | 0.08 | 1.37  |
| Mean weight gained (g)      | 8.95   | 8.95   | 14.41  | 11.37  | 15.70  | 11.88  | 3.10 | 1.39  |
| Percentage. weight gain (%) | 134.64 | 137.27 | 231.30 | 174.39 | 235.74 | 182.67 | 1.86 | 21.93 |
| Specific Growth rate (%)    | 0.53   | 0.53   | 0.73   | 0.63   | 0.74   | 0.63   | 0.10 | 0.05  |
| Total Feed fed (g)          | 0.45   | 0.47   | 0.67   | 0.55   | 0.81   | 0.59   | 0.01 | 0.07  |
| Feed conversion ratio       | 4.28   | 4.26   | 3.24   | 3.73   | 3.50   | 3.80   | 0.01 | 0.21  |
| Protein Intake              | 18.25  | 18.93  | 29.49  | 22.82  | 33.64  | 24.63  | 0.48 | 3.01  |
| Protein Efficiency ratio    | 0.49   | 0.47   | 0.49   | 0.49   | 0.47   | 0.48   | 0.01 | 0.00  |
| Net protein Utilization     | 15.01  | 13.73  | 17.94  | 16.83  | 17.89  | 16.28  | 0.91 | 0.83  |
| Average Survival (%)        | 95     | 94     | 97     | 94     | 96     | 95.2   | 1.30 | 0.58  |

key word: *Moringa*, steamed, *Clarias gariepinus*

## **CURRICULUM DEVELOPMENT AT POSTGRADUATE LEVEL: EXAMPLES IN AQUACULTURE**

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Curriculum development is a planned, purposeful, progressive and systematic process for creating improvements in education and this process evolves with changing times. It has a broad scope which encompasses the educational institutions, the learners, the teachers and the development of society in general. Curriculum development provides solutions to humanity's pressing problems such as environment, socio-economics, poverty, climate change and sustainable development. At postgraduate level, curriculum development is more engaging in terms of leadership, internationalization, stakeholder involvement, knowledge of the students' entering behavior, the stock of resources to be invested (human skills, financial, infrastructure, etc), market demand/need for the curriculum, acceptability beyond the implementing institution and country, relationship to other disciplines, and the anticipated contribution to current and future clienteles. It is a process that requires cooperation, collaboration, independence as well as dependence for successful output.

Curriculum development involves structuring of the learning offerings that stride on three main dimensions of human development: the cognitive, affective and psychomotor. When these three dimensions are appropriately planned for in the curriculum and implemented, the clients/students benefit at optimal levels. Curriculum development at all levels has definite steps that are interrelated and often are addressed consecutively and concurrently for effective education. For purposes of discussion, these steps include: problem identification and general needs assessment, needs assessment of the target students, goals and objectives, educational strategies, implementation, and evaluation and feedback. The whole process is cyclic and continuous due to the dynamic nature of education and life in general.

This paper discusses the intrigues involved in developing curriculum at postgraduate level using examples in Aquaculture. It provides benchmarks that should be taken care of before the curriculum development output can receive acceptance within the countries concerned, the region and across regions. Such acceptance is important for internationalization of curriculum.



## EVALUATION OF FORMULATED FEED OF PLANT AND ANIMAL ORIGIN ON THE GROWTH PERFORMANCE OF *Oreochromis niloticus* IN PONDS, ETHIOPIA

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The present study was conducted to evaluate the effect of supplementary feeding with only plant material origin (Noug cake, Brewery waste & wheat bran) and mixture of plant and animal origin (Fish meal, Brewery waste & wheat bran) with similar protein content of two formulated feeds on the growth of *Oreochromis niloticus* in concrete ponds.

The feeding trial was conducted in six concrete ponds of about 50 m<sup>2</sup> each. The control and experimental groups were run in duplicate with stocking density of 2 fish/m<sup>2</sup>. The treatment groups were fed at 5% of their body weight with the respective test feeds and the control group was left without any supplementary feed. The results of the experiment showed that fish given supplemental diets grew much faster (ANOVA,  $P < 0.05$ ) than the control group. Although no significant growth differences were observed between the test feeds (ANOVA,  $p = 0.42$ ), fish raised in mixture of plant and animal origin (0.35 g/day) showed better growth rate than plant origin. Differences in growth rates between the control and test feeds as well as among the test feeds can be attributed to both direct and indirect effects of supplementary feeds given to the fish. Direct intake of feeds by the fish provides more nutrients resulting in better growth and production. Therefore, all supplementary feeds have an impact on the growth of *O. niloticus* in ponds.

### Biography

Mohammed oumer has completed his BSC at the age of 21 years from Haromaya University and MSC from Bahir dar University School of agriculture. He is the aquaculture researcher of Bahir dar fisheries and other aquatic life research center. He has published 3 papers in reputed journals and has been serving as an aquaculture case team coordinators.

## IDENTIFYING DISEASE HAZARDS AND DETERMINING RISKS AND IMPACTS OF DISEASE ON AN AQUACULTURE OPERATION

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The aquaculture producer is likely familiar with diseases that cause problems on the farm. However, it is possible that not all of those diseases are considered a “serious potential hazard”. Therefore, producer and the attending veterinarian should obtain information from a regulatory authority of the region/country about the current epidemiological situation of important or reportable diseases that could be of concern to the farming operation. When a list of diseases that would be hazardous to the Epidemiological Unit (EpiUnit) is identified, their respective risks and impacts need to be determined.

A number of different approaches can be used to perform qualitative or quantitative risk assessments. In most cases semi-quantitative approaches are adequate for developing a farm-based biosecurity plan. In the OIE Aquatic Animal Health Code disease risks are classified based on several criteria so that each disease can be evaluated and ranked according to its potential to cause production loss and possible spread to other areas or animals in the wild. Additional criteria are based on the availability of reliable diagnostic tests used to definitively identify any disease in question. It is important to note that not all diseases of concern for every farm or EpiUnit fulfil the requirements for being listed as reportable diseases with OIE and that each country or local area might regulate diseases and require control measures depending on the epidemiology and the impact of a disease on their local production impact or trade situation. Therefore, each EpiUnit (country, region, farm etc.) has to be evaluated based on its own specific situation, and the need to identify which diseases pose specific hazards and have the highest probability of occurring. Such identification and probability estimation may be done by risk profiling. Using a relatively simple semi-quantitative approach, the risk of each disease to a farm can be defined through estimating the probability of it occurring and the consequences of the occurrence (i.e. risk = probability x consequences). This approach can provide objective information on the risk associated with a specific hazard (an infectious disease in this case).

Using available information (disease reports and epidemiological information available) and expertise including associated variability and uncertainty, the risk assessment process will provide insight on various steps needed to prevent the hazardous diseases being introduced or released from the farm or EpiUnit. During this step of developing a biosecurity program, the relative “weight” for each identified disease needs to be determined based on the experience of the producer and veterinarian, with possible input from the government. Once the disease hazards are prioritized, the next step would be to evaluate critical points on the farm or in the farms management where these diseases may enter or leave the EpiUnit, and decide on best applicable preventive measures.

## EFFECTS OF MODIFIED ZEOLITE (CLINOPTILOLITE) USED AS FEED ADDITIVE ON PRODUCTION PARAMETERS IN SHRIMP AND FISH AQUACULTURE

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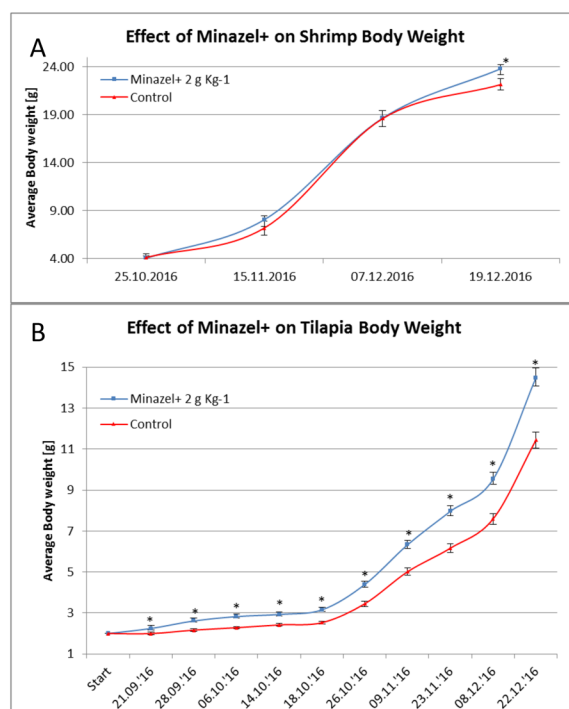
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Zeolites have been used as animal feed additive to prevent mycotoxin effects in different species, including aquatic animals, as mycotoxin contamination of feed and feed ingredients used in aquaculture is an ongoing global problem. Recently, a novel process of chemical modification of zeolite (clinoptilolite E567/568; Minazel+®) surface with addition of organic cations has been shown to increase selective adsorption of both polar and non-polar mycotoxins in contaminated feed. However, the safety and effects of this new additive on fish and shrimp health and production parameters have not yet been studied. We report the effects of Minazel+ used as feed additive (2 g Kg<sup>-1</sup>) in commercial diets fed to healthy freshwater fish fingerlings (Nile tilapia, *Oreochromis niloticus*; and Common carp, *Cyprinus carpio*), and to healthy pacific white legged shrimp (*Litopenaeus vannamei*) for 50-90 days.

The fish were reared in recirculation systems with automated 30% water exchange per day. Two hundred fish of each species were randomly assigned to treatment (with Minazel+®) and experimental (no additive) group. Shrimp (200/group) were housed in two 1,000 liter tanks connected to a large commercial shrimp production recirculation system (Crustanova®). The fish and shrimp were sampled on regular basis to collect biometric parameters (Fig. 1.), perform clinical health and histopathological exam, and total and differential leukocyte counts.

All experimental groups showed significant increase in body weight and decrease in food conversion ratio. Clinical and pathological findings were not significantly different among treatment and control groups. We conclude that addition of 2 g Kg<sup>-1</sup> of Minazel+® to commercial fish and shrimp diets increased production parameters in three major aquaculture species without observable side effects.

Figure 1. Effects of Minazel+® additive (2g Kg<sup>-1</sup>) on body weight on A) Pacific white legged shrimp; and B) Nile tilapia; \*denotes significant differences at  $p < 0.05$ .



## ECHINOCULTURE IN GREECE: PRESENT STATUS & PERSPECTIVES

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### Introduction

Sea urchins are considered a delicacy world wide and quite nutritious as the urchin roe (eggs) has a high percentage of carotenoids, fatty acids, vitamins and other anti-oxidant agents. Various edible species in Europe and around the world achieve good prices in European or Asian markets (Pantazis 2009; Kelly & Chamberlain 2010). A Presidential Decree signed on 28.02.2014 by the Greek Government, has regulated the sea urchin fishery in Greece in order to secure its sustainability [<http://www.minagric.gr/index.php/el/home-2/89-greek-content/2010-07-14-10-03-42/grafeiotypou/press/2218-dt280214a>]. It is obvious that the sea urchin fishery in Greece is threatened by over-depletion of the wild stocks and therefore there is a need for a more systematic culture approach of the species.

### Materials and Methods

In 2013 the Innovation and Business Unit (IBU) of the University of Thessaly [[www.moke.uth.gr](http://www.moke.uth.gr)] has decided to investigate the potential of sea urchin culture in Greece and provide an appropriate business plan for its development. Based on international experience and national research experience (Pantazis et al. 2008; Pantazis 2009) and by recruiting experienced business developers and financial advisors, the IBU has produced a sound business plan with the title “Integrated and Sustainable Sea Urchin culture in Greece”. This business plan has been adopted by various entrepreneurship events and industrial organizations [<http://sev4enterprise.org.gr/epixeirimatikotita/>]. On January 2016, this sea urchin development plan has been awarded a special distinction at the Blue Growth Event [<http://www.protothema.gr/city-stories/article/546432/kainotomotas-sti-thalassa/>] organized by the “Blue Growth” Innovation hub [<http://bluegrowth.gr/>], the municipality of Pireaus, Greece and the Aeuthoria net [<http://www.aeuthoria.net/>].

### Results and Discussion

During the summer in Greece, sea urchin eggs are sold in restaurants at a retail price of 6-7 euros per ration [[http://mychania.blogspot.gr/2007/01/blog-post\\_4235.html](http://mychania.blogspot.gr/2007/01/blog-post_4235.html)], which equals to approximately 40 euros per kilo of fresh sea urchins. An extensive investigation at various local restaurants in Crete, Central Kyclades and Magnissia prefectures has revealed that a commercial sea urchin production unit could easily distribute 100 tons of fresh sea urchins annually in the Greek markets. On the other hand there are no sea urchin aquaculture producers in Greece. The existing aquaculture Greek fish producers do not fall within the “competitors’ category” as they produce animal protein to meet the basic edible needs of human population, whereas sea urchins are a luxury food, addressing niche and specialized markets (seafood restaurants, oriental cuisine, catering for air carriers and sea cruisers, luxury hotels, etc). The business team consists of specialized scientists which hold the know-how for the integrated sea urchin production. Furthermore, the location of the farm guarantees the provision of good quality seawater and the viability of the selected sea urchin species (*Paracentrotus* spp. *Echinus* spp.), as they are endemic and already found in this geographical area, characterized by large natural populations. The farm location guarantees the market distribution of the product at a reasonable cost. In addition the production process will utilize the concept of Integrated Multi-Trophic Aquaculture (IMTA) which minimizes the environmental fingerprint of this aquaculture operation. Last but not least, are the ideal meteorological conditions of the area which guarantee the achievement of the commercial size of the final product at a reasonable production time frame.

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## ESTIMATION OF GENETIC PARAMETERS FOR HIGH WATER TEMPERATURE TOLERANCE IN PACIFIC ABALONE, *Haliotis discus hannai*

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Pacific abalones are commercially important marine resources in eastern Asia. In Korea, 6 species of abalone are naturally distributed along the coast: *Haliotis discus hannai*, *Haliotis discus discus*, *Haliotis madaka*, *Haliotis gigantea*, *Haliotis diversicolor diversicolor*, and *Haliotis diversicolor supertexta*. Of these, *H. discus hannai* represents one of the most valuable fishery resources. It is a highly sought-after delicacy, the production of which has been increasing dramatically by about 1,000 t/y since 2003, reaching 13,513 t in 2016. Thus abalone aquaculture in Korea has been dominated by *H. discus hannai*. However, mass mortality resulting from acute water temperature stress has posed a serious threat to the sustainable development of the abalone aquaculture industry. Therefore, a project to develop a breeding program for environmental adaptation is being carried out.

To test variation in high water temperature tolerance between abalone families, 52 abalone families produced using single-pair mating design and these were reared in same tank. At 15 months, high water temperature stress experiment was performed to obtain the basic data on estimation of genetic parameters for high water temperature tolerance trait using 52 abalone families. The 1,559 progeny were successfully assigned to their parents based on shared alleles at 10 polymorphic microsatellite loci. The survival rate of each family ranged from 80.5 % to 3.3% (Figure 1.). Using an animal model, heritability estimates was 0.228 for high water temperature tolerance trait. Genetic correlations between growth and high water temperature tolerance were low ( $\leq 0.45$ ), indicating that breeding for high water temperature tolerance has nothing to do with growth. Thus, to develop of abalone with high water temperature tolerance trait, breeding program for growth improvement must be carried out separately.

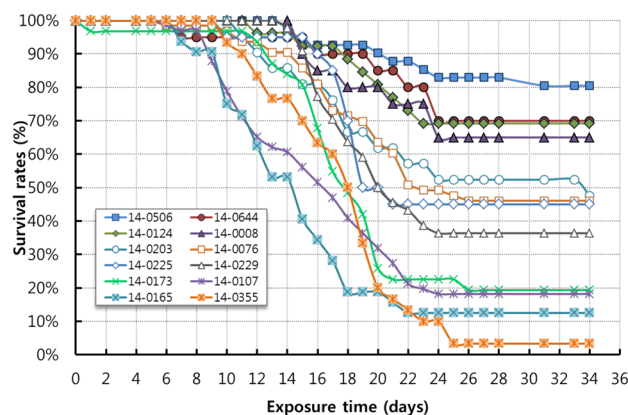


Figure1. Survival rates of the abalone, *Haliotis discus hannai*, exposed to high water temperature

## EFFECTS OF THE FEED ADDITIVE OF MARINE MICROORGANISM CONTAINING PIGMENT ON BODY COLOR AND GROWTH OF *Epinephelus akaara*

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The red body color is considered an important factor which determines the market price of the fish, and farmers use carotenoid-added feed to improve the red color. It is known that carotenoids are not generated within the fish and accumulated in the fish body by eating carotenoid-added feed.

However, to be added into fish feed, these pigments should be first refined and this process places a high cost burden the farm operation. Therefore, in this study, we added natural astaxanthin, a carotenoid cheaply made from marine microorganism *Paracoccus haeundaensis* along with *Lactobacillus heveticus* into fish feed and this diet was directly provided with the fish to improve the body color at a lower cost.

Depending on the pigment concentration and existence of the lactic acid bacteria, 6 experiment groups were designed (the control group with not pigment addition, the experiment groups of *P. haeundaensis*-derived astaxanthin 100, 1,000 and 3,000 ppm and the experiment groups of *P. haeundaensis*-derived astaxanthin 100 and 1,000 ppm mixed with *L. helveticus*). Triplicate experiments were conducted for 90 day rearing. Conclusions from the experiments proved that the addition of pigment or lactic acid bacteria does not significantly affect the growth and survival of *E. akaara*. According to color measurements of the skin and muscular by a colormeter, the a value indicating skin redness was higher in the experimental groups than the control while no significant difference in the muscle color was not observed between the experiment and control groups.

## **PREVENTION OF KOI HERPES VIRUS DISEASE ON GOLD FISH WITH PELLETT PLUS IG-Y ANTI KOI HERPES VIRUS**

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Koi Herpes Virus(KHV) has been associated with devastating losses of common carp (*Cyprinus carpio carpio*) and ornamental (koi) carp (*Cyprinus carpio*) in Java and Sumatra island. The first outbreak in Koi was reported in Blitar, East Java then spread to the common carp culture in West Java province, Lubuklinggau (southeast sumatra province), Maninjau lake (west sumatra province) and Toba lake (north sumatra province). Total economics losses since 2002 up to 2004 approximately twenty five million US\$. The novel anti-KHV strategies are urgently needed. In this report, KHV particles were concentrated and then used for the immunization of hens in order to obtain the anti-KHV egg yolk immunoglobulin (IgY). Yolk egg (IgY) were mixed in pellet and given to two group of common carp, one group was given for 14 days and the other group for 28 days. After oral passive immunization with IgY, both groups were challenged with KHV field isolate. The results showed that mortality rate in vaccinated group (30%) significantly lower compared to those of unvaccinated group (90%). These results will possibly pave the way for the prevention of KHV infection



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In order to determine the current status of fish farming in Thulamela 20 smallholder fish farmers were interviewed based on a project funded by the Water Research Commission entitled “Knowledge transfer on water resource management for improved integrated aquaculture and agriculture systems”. The questionnaire focused on three main areas: Profile of the fish farmer, problems faced, and knowledge sources used. The responses were analysed using general statistics and descriptive text.

- Freshwater fish farming as a thriving commercial enterprise is virtually non-existent and isolated fish farmers are doing their own thing in the best way that they can manage.
- Fish farming is mainly a subsistence activity.
- Farmers desire extension services to assist in practical ways to address barriers to production.
- Small ponds, limited access to quality seed and high feed costs and availability present major stumbling blocks to aquaculture development.

- Ponds are gradually increased in number and size.
- Government subsidises quality feed and seed.
- Farmers operate around clusters in nodal points (Fig. 1).
- The produce from these clusters are absorbed by an aquaculture development zone in this region.

A map of the Maseru District showing various villages and their proximity to perennial water sources. The map includes labels for villages such as Meaneta, Mhanzwi, Makende, Tshenelo, Tsifudi, Knubu, Thohoyandeni, Thohoyandou, Gondeni, Sibasa, Muledhe, Phophidi, Vondo, Khalavha, Tshin-vha, Dopeni, Mandala, Shanzha, Tshifularani, and Lwanando. A legend box indicates that Perennial water accounts for 75% and Shortages Winter accounts for 25%. Another box at the bottom right states the average distance to the Agricultural Office is 26 km. The map also shows major roads like N1 and R102, and geographical features like the Orange River.

Fig.1 Smallholder farmers visited are clustered around nodal points (red)

## **GUTS TO GLORY: CONVERSION OF RAINBOW TROUT *Oncorhynchus mykiss* VISCERA TO FEED OIL FOR USE IN DIETS OF AFRICAN CATFISH (*Clarias gariepinus*)**

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Aquaculture waste streams from Southern African processing facilities are currently not optimally utilized. Waste streams such as gills, viscera and frames are either collected from processing facilities by animal protein renderers, pig farmers for use as a nutrient source or, as with mortalities, discarded at landfill sites. Here the leachates and emissions resulting from their spoilage may impact negatively on human health, food safety and environmental pollution and more solutions are sought to promote responsible practices throughout the aquaculture value chain to minimise environmental degradation, while simultaneously developing a sustainable value chain.

In this study, rainbow trout viscera were ensiled using two acid preservation methods (Formic acid x Lactic acid; Propionic acid x Lactic acid) and one microbial preservation method (bacterial blend x sugarcane molasses). The aim of the study was to establish 1) efficiency of preservation of the various silage preparation methods in terms of microbial stability, 2) separation efficiency of differential oil and protein hydrolysate phases 3), and application of oil phases as fish oil alternatives in diets of African catfish.

## SEQUENCING AND MALDI TOF - NEW DIAGNOSTIC METHODS APPLIED TO IDENTIFY THE BACTERIA ISOLATED FROM DISEASED FRESHWATER FISH

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Identification of bacteria causing infections in fish is difficult, mainly due to the lack of specific diagnostic tests intended for fish pathogens. Routinely, the identification of the bacteria is carried out biochemically, using commercial available tests. Unfortunately, these assays are dedicated mainly to human/mammalian pathogens, therefore incorrect identification results often appeared.

Some serological methods like agglutination or ELISA are also applied, but their importance is limited. However, new and specific diagnostic techniques are still searched. Sequencing and MALDI-TOF are those methods, which are very promising in proper bacterial identification.

This research was supported by KNOW (Leading National Research Centre) Scientific Consortium “Healthy Animal - Safe Food”, decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

In this study a total of 170 different bacterial isolates collected from freshwater fish and initially identified biochemically as *Aeromonas* spp., *Burkholderia cepacia*, *Chryseobacterium* spp., *Myroides* spp., *Pantoea* spp., *Plesiomonas shigelloides*, *Pseudomonas oryziatrans*, *Shewanella putrefaciens*, *Ps. fluorescens/putida*, *Stenotrophomonas maltophilia*, *Yersinia ruckeri*, *Weeksella virosa* were used. Preliminary identification results were then verified by sequencing and MALDI TOF.

PCR product from the bacterial conservative region 16S rDNA were used to sequencing. Identification by MALDI-TOF using Bruker's apparatus and databases was also performed.

The performed preliminary study showed the usefulness of both sequencing and MALDI-TOF to identify the bacteria isolated from fish (Table 1). The big advantage of MALDI TOF is time saving without losing accuracy.

| Biochemical identified                                 | MALDI TOF  | Sequencing   |
|--|--|--|
| <i>Aeromonas sobria</i>                                | <i>Aeromonas</i> sp.                                   | <i>Aeromonas veronii</i>                           |
| <i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> | <i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> | <i>Aeromonas salmonicida</i> s. <i>salmonicida</i> |
| <i>Burkholderia cepacia</i>                            | <i>Aeromonas</i> sp.                                   | <i>Aeromonas hydrophila</i>                        |
| <i>Citrobacter youngae</i>                             | <i>Citrobacter</i> sp.                                 | <i>Citrobacter freundii</i>                        |
| <i>Comamonas testosteroni</i>                          | <i>Pseudomonas alcaligenes</i>                         | <i>Pseudomonas alcaligenes</i>                     |
| <i>Myroides</i> spp.<br><i>Weeksella virosa</i>        | <i>Brevundimonas diminuta</i>                          | <i>Brevundimonas diminuta</i>                      |
| <i>Pasteurella multocida</i>                           | <i>Chryseob. yeoncheonense</i>                         | <i>Chryseob. koreense</i>                          |
| <i>Plesiomonas shigelloides</i>                        | <i>Plesiomonas shigelloides</i>                        | <i>Plesiomonas shigelloides</i>                    |
| <i>Pseudomonas fluorescens</i>                         | <i>Pseudomonas fluorescens</i>                         | <i>Pseudomonas fluorescens</i>                     |
| <i>Pseudomonas luteola</i>                             | <i>Acinetobacter</i> spp.<br><i>Hafnia alvei</i>       | <i>Acinetobacter parvus</i><br><i>Hafnia alvei</i> |
| <i>Pseudomonas oryziatrans</i>                         | <i>Acinetobacter lwoffii</i>                           | <i>Acinetobacter lwoffii</i>                       |
| <i>Shewanella putrefaciens</i> group                   | <i>Shewanella putrefaciens</i>                         | <i>Shewanella putrefaciens</i>                     |
| <i>Stenotroph. maltophilia</i>                         | <i>Stenotroph. rhizophila</i>                          | <i>Stenotroph. rhizophila</i>                      |
| <i>Sphingomonas paucimobilis</i>                       | <i>Flavobacterium</i> sp.                              | <i>Flavobacterium</i> sp.                          |

TABLE 1. Comparison of diagnostic methods used to bacterial identification.

## USE OF FIVE ANAESTHETICS DURING HANDLING AND CONFINEMENT OF CLOWN ANEMONEFISH *Amphiprion ocellaris*

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The clown anemonefish *Amphiprion ocellaris* is the most popular fish species in world aquarium trade. The demand increases the necessity of new technology to supply the market with healthy captivity animals. To guarantee this quality, mitigation procedures of stress caused during the production process are required. The aim of this study was to evaluate the effectiveness of five different anaesthetics during handling and confinement condition similar to those used to transportation of *A. ocellaris*.

Initially, fish were exposed individually for 15 minutes to five different concentrations of MS-222, propofol, and camphor, clove and mint essential oils ( $n=10/\text{concentration}/\text{anaesthetic}$ ). The anaesthesia stages were verified, being the goal stage IV (surgical anaesthesia). Posteriorly, animals suffered biometrics and were transferred to clean saltwater for anaesthesia recovery. In a second test, animals were randomly selected and immersed in plastic bags containing saltwater and pure oxygen (1:2 ratio) plus anaesthetic. Three concentrations of each product ( $n=8 \text{ fish/time/concentration}$ ) were analyzed after 6, 12 and 24 h of confinement. Dissolved  $O_2$ , pH, total ammonia were measured before closing and after opening the bags. Finally, the influence of different densities (5, 10, 15 and 20 fish  $L^{-1}$ ) in confinement conditions were evaluated during 24h, under the effect of the ideal anaesthetics concentrations of previous test. The same water quality parameters were monitored, including dissolved  $CO_2$ .

Based on anaesthetic induction and recovery times, mortality of exposed animals and the improvement in water quality in confinement condition, the ideal concentration of each anaesthetics were defined (Table 1). The water quality remained in acceptable limits along the different times of confinement in all treatments, except in control. MS-222, mint, clove and camphor oils significantly reduced the concentration of N-TA and  $CO_2$  after 24h confinement in different densities, comparing to control group. Propofol has provided less improvement in water quality than others, but fish were safely sedated at 0.3 mg  $L^{-1}$ .

TABLE 1. Ideal concentrations and performance of anaesthetics during handling and 24 h transport of clown anemonefish *Amphiprion ocellaris*.

| Anaesthetic | Handling           |           |          | Transport          |              |
|-------------|--------------------|-----------|----------|--------------------|--------------|
|             | Ideal              | Induction | Recovery | Ideal              | Fish Density |
|             | [ ]                | (s)       | (s)      | [ ]                | ( $L^{-1}$ ) |
| Camphor     | 500 $\mu L L^{-1}$ | 535.0     | 229.0    | 120 $\mu L L^{-1}$ | 05           |
| Clove       | 27 $\mu L L^{-1}$  | 310.5     | 396.0    | 05 $\mu L L^{-1}$  | 05           |
| Mint        | 70 $\mu L L^{-1}$  | 312.0     | 329.5    | 25 $\mu L L^{-1}$  | 10           |
| MS-222      | 80 mg $L^{-1}$     | 353.8     | 255.0    | 15 mg $L^{-1}$     | 15           |
| Propofol    | 0.7 mg $L^{-1}$    | 280.0     | 1,507    | 0.3 mg $L^{-1}$    | 10           |

## EFFECT OF TEMPERATURE ON LARVAL AND POST-LARVAL ONTOGENIC DEVELOPMENT OF *Astyanax altiparanae* IN LABORATORY CONDITIONS

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Tetra *Astyanax altiparanae* Garutti & Britski (2000) has gained the attention of researchers because, besides being increasingly used in commercial aquaculture in Brazil, has biological characteristics considered ideal for its use as a model species in laboratory fish trial, such as xenobiotic exposure tests, mutagenicity tests and biological experiments in general. In this work, the initial development of the species was accompanied under three different temperatures (20, 25 and 30°C). The monitoring was carried out from the moment of hatching larvae in order to characterize the effects of temperature on larval and post-larval development and survival. The 48 larvae for each tested temperature were individually monitored. The ontogenetic monitoring was recorded in post-hatching hours (hpe) and post-hatching day (dpe). The larval and post-larval was divided into four stages (I - Larval-yolk II - Pre-bending, III - bending of the notochord, IV - Post-bending). Total length (TL), body height (HC1), head length (CC), head height (HC2), hole diameter (OD), yolk sac length (CV), the yolk sac height (HV) and yolk reserve volume (V) were daily measured.

There was large variation in the occurrence of ontogenetic events related to temperature. The higher the temperature, the faster the development processes and the larger allometric growth rates were registered. None of the individuals at 20°C and 30°C completed the post-larval development, surviving only to the stage II (20°C) and III (30°C).

Only at the temperature of 25°C was possible to reach 8% survival rate in stage IV, indicating that this is the temperature most suitable for *A. altiparanae* laboratory larval rearing.

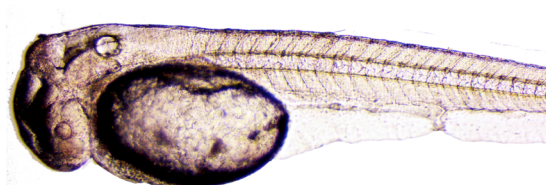


Fig 1. *A. altiparanae* during the yolk-sac larval stage.

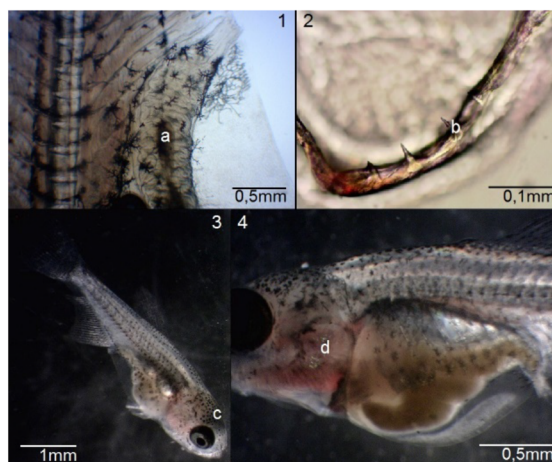


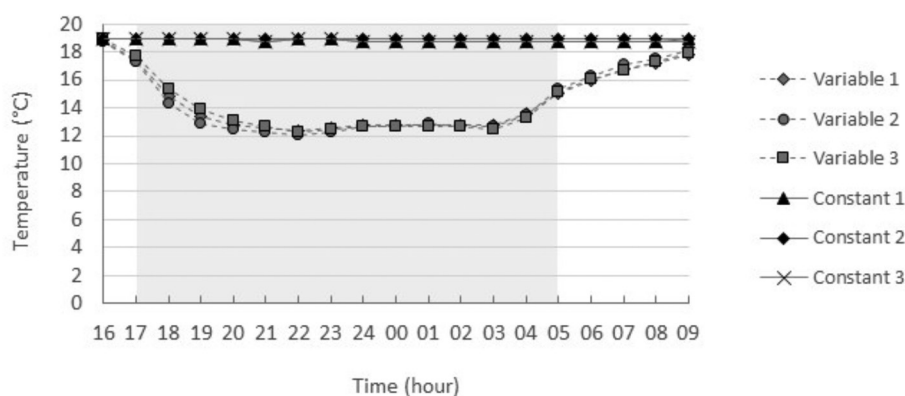
Fig 2. *A. altiparanae* in the final stage of larval ontogenetic development: (1) presence of food in the digestive tract (A); (2) teeth (B); (3) punctate pigmentation on the dorsal region of the head (C); and (4) scales (D).

## PHYSIOLOGICAL RESPONSE OF GILTHEAD SEABREAM *Sparus aurata* EXPOSED TO LONG-TERM DAY/NIGHT TEMPERATURE VARIATIONS

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Temperature is one of the most important factors affecting the growth and development of fish, since it influences body functions like food consumption, feed conversion metabolism, growth rate, affecting the overall fish performance. In a fish farm, heating water can reduce the fish production cycle but can become extremely expensive, compromising firm viability. Solar energy technologies may provide affordable energetic solutions to bypass this obstacle by heating water at least during the day period, whereas during the night water temperature attains environmental values. Although fish is tolerant to water temperature oscillations, this daily pattern might result in an effective stressor, where fish undergo a series of biochemical and physiological changes to cope to the new rearing conditions. The aim of this study was to evaluate juvenile gilthead seabream (*Sparus aurata*) response to day/night water temperature variation over a 60 days period, by monitoring several physiological parameters: biochemical, growth, histological and molecular. Fish ( $96.3 \pm 1.0$ g body weight) were exposed to cold water from 5pm to 5am with a temperature variation of  $7.5 \pm 0.19^\circ\text{C}$  (Fig1). The water temperature of the constant group was maintained constant ( $20.6 \pm 0.03^\circ\text{C}$ ). Our results revealed that variable treatment showed interesting changes on fish metabolism.



**Figure 1.** Daily water temperature variation of constant and variable treatments. The shaded area represents the water-cooling period (5pm to 5am).

This work was undertaken in the framework of AQUAENERG project (31-03-05-FEP-0076) supported by PROMAR Program (Portugal).



## EFFECT OF FEEDING FREQUENCY ON GROWTH AND ENZYMATIC ACTIVITY OF *Litopenaeus vannamei* IN BIOFLOC SYSTEM

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The present study evaluated the zootechnical performance and enzymatic activity of *Litopenaeus vannamei* reared at different feeding frequencies during the nursery phase in biofloc system.

The experiment consisted of four treatments, corresponding to the feeding frequencies of 1, 2, 3, and 4 times a day. Twelve-day postlarvae (PL12) were stocked in 12 circular tanks at a density of 3000 m<sup>-2</sup> for 35 days. These tanks were connected to a recirculation system supplied by a matrix tank where biofloc management was carried out. Water quality remained within acceptable limits for the species over the experiment.

Food frequencies had no influence on survival (88.5 to 92.7%) and feed conversion ratio (1.5 to 1.7), but the final mean weight (0.43 to 0.56g) was significantly higher in shrimp fed three times a day (Table 1). This fact is probably associated with amylase and trypsin activities, as well as the significant increase of chymotrypsin and lipase in shrimp of this treatment at the end of culture period. Feeding three times a day provided the highest enzymatic activity and the best zootechnical performance of *L. vannamei* during the nursery phase in biofloc system.

Table 1. Zootechnical performance (mean  $\pm$  SD) of *Litopenaeus vannamei* reared for 35 days at different feeding frequencies (1 to 4 times a day) during the nursery phase in biofloc system.

|                  | 1x                            | 2x                            | 3x                            | 4x                            |
|------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Final weight (g) | 0.44 $\pm$ 0.05 <sup>a</sup>  | 0.43 $\pm$ 0.08 <sup>a</sup>  | 0.56 $\pm$ 0.15 <sup>b</sup>  | 0.43 $\pm$ 0.12 <sup>a</sup>  |
| Survival (%)     | 92.71 $\pm$ 2.64 <sup>a</sup> | 90.87 $\pm$ 2.61 <sup>a</sup> | 88.51 $\pm$ 1.15 <sup>a</sup> | 89.84 $\pm$ 3.24 <sup>a</sup> |
| FCR              | 1.76 $\pm$ 0.21 <sup>a</sup>  | 1.75 $\pm$ 0.27 <sup>a</sup>  | 1.50 $\pm$ 0.46 <sup>a</sup>  | 1.67 $\pm$ 0.37 <sup>a</sup>  |

Different superscript letters in the same row represent significant difference ( $p < 0.05$ ).

FCR = Food conversion ratio.



## MICROFLORA ISOLATED FROM SKIN ULCERS IN COD *Gadus morhua* FROM THE SOUTHERN BALTIC SEA

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Skin ulcerations is the most serious and frequently observed diseases in the Baltic cod (*Gadus morhua*). Etiological agents of these disorders in fish may be different. It is generally known that ulceration process is caused by an opportunistic bacteria, which might be also responsible for causing different clinical syndromes, serious diseases, and even bacteremia in human. The clinical condition of the fish, including ulcer development may be closely related to the conditions of the environment, in particular with poor water quality. Various aspects of the ecosystem of the southern Baltic Sea was investigated during research surveys in April 2016, including fish condition and their health status in terms of prevalence of skin ulcers in cod. Tissue samples of the cod exhibiting pathological symptoms were collected. Classical bacteriological methods as well as molecular techniques were used to bacteria identification.

This research was supported by The National Centre for Research and Development under the Strategic Program Biostrateg II (grant no. 296211/4/NCBR/2016).

Total number of about 100 fish exhibiting skin ulceration (Ryc. 1) were collected from five different areas of the Baltic Sea (Bornholm S, Bornholm N, Kolobrzeg-Darlovo, Slupsk Furrow, and Gulf of Gdansk). Additionally, in order to microflora comparison, thirty clinically healthy individuals from each sampling points was included.

More than 500 bacterial strains were isolated. The prevalence of bacteria in diseased and clinically healthy fish was calculated (Table 1). Bacteria *Aeromonas* spp., *Acinetobacter* spp., *Chryseobacterium* spp., *Pseudomonas* spp., *Shewanella putrefaciens* were found as the dominant species isolated from ulcers. From apparently clinically healthy fish, mainly *Aeromonas* spp. and *Shewanella putrefaciens* were isolated.

Ryc. 1 Skin ulcers in cod.



TABLE 1. The prevalence of bacteria isolated from cod.

| Bacteria isolates       | <i>Aeromonas</i> spp | <i>Pseudomonas</i> spp | <i>Shewanella putrefaciens</i> | Other |
|-------------------------|----------------------|------------------------|--------------------------------|-------|
| Skin ulcer              |                      |                        |                                |       |
| Prevalence              | 20%                  | 20%                    | 35%                            | 25%   |
| Clinically healthy fish |                      |                        |                                |       |
| Prevalence              | 40%                  | 3%                     | 55%                            | 2%    |

## EXOGENOUS METABOLITES BOOST CENTRAL METABOLISM TO KILL ANTIBIOTIC-RESISTANT BACTERIA

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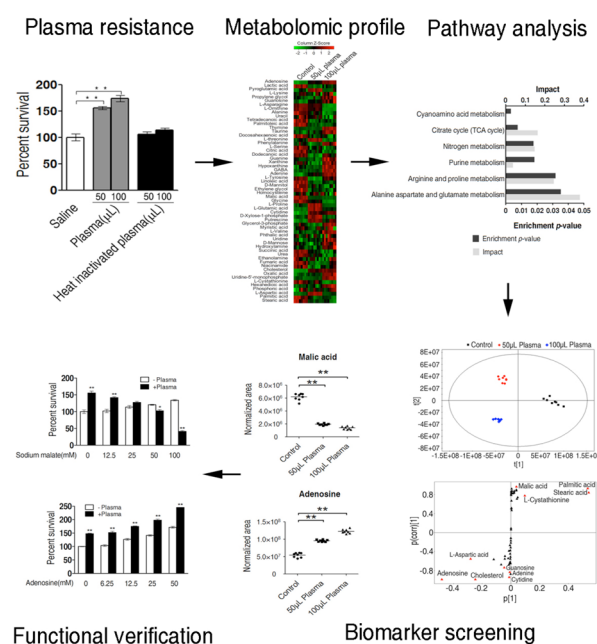
Multidrug-resistant bacteria are an increasingly serious threat to human and animal health. However, novel drugs that can manage infections by multidrug-resistant bacteria have proved elusive. Here we show that glucose and alanine abundances are greatly suppressed in kanamycin-resistant *Edwardsiella tarda* by GC-MS-based metabolomics. Exogenous alanine or glucose restores susceptibility of multidrug-resistant *E. tarda* to killing by kanamycin, demonstrating an approach to killing multidrug-resistant bacteria. The mechanism underlying this approach is that exogenous glucose or alanine promotes the TCA cycle by substrate activation, which in turn increases production of NADH and proton motive force and stimulates uptake of antibiotic. Similar results are obtained with other Gram-negative bacteria (*Vibrio parahaemolyticus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) and Gram-positive bacterium (*Staphylococcus aureus*), and the results are also reproduced in a mouse model for urinary tract infection. This study establishes a functional metabolomics-based strategy to manage infection by antibiotic-resistant bacteria.

# METABOLOMICS APPROACH FOR INVESTIGATION AND CONTROL OF SERUM-RESISTANT BACTERIA IN AQUACULTURE

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Serum resistance has been shown in numerous bacterial systems to be critical for the survival of certain invading bacteria and the establishment of infectious disease, which is dependent on competing action of complement system. Control of serum resistance is an ideal approach to eliminate bacterial pathogens. Reprogramming metabolome is an effective, economic and green way to combat the invaders. These motivated us to investigate bacterial metabolome contributes to the resistance. Taking *Streptococcus agalactiae* for an example, we established a pathogen-plasma interaction model by which we explored how *S. agalactiae* evaded serum-mediated killing. We found that *S. agalactiae* grew faster in the presence of yellow grouper plasma than in the absence of the plasma, indicating *S. agalactiae* evolved a way of evading fish immune system. To unveil the underlying events behind this phenotype, we applied GC-MS based metabolomics approaches to identify differential metabolomes between *S. agalactiae* cultured with and without yellow grouper plasma. Through bioinformatics analysis, decreased malic acid and increased adenosine were identified as the most crucial metabolites that distinguish the two groups. Meanwhile, they belonged to decreased TCA cycle and elevated purine metabolism, respectively. Finally, exogenous malic acid and adenosine were used to reprogram the plasma-resistant metabolome, leading to elevated and decreased susceptibility to the plasma, respectively. Therefore, our findings reveal the first time that *S. agalactiae* mounts metabolic trick to response plasma killing as a result of serum resistance, which may be reverted and enhanced by exogenous malic acid and adenosine, respectively, suggesting that the metabolic trick can be regulated by metabolites. Our approach is also demonstrated in other pathogens including *Edwardsiella tarda* and *Vibrio fluvialis*.



## MARKETING CHANNELS UTILISED BY TILAPIA PRODUCERS IN SOUTH AFRICA

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This paper examines the choice/variety of market channels used by tilapia growers in South Africa. In the South African context this is an important element for farmers because they are challenged by competitively priced imports from Asia, whilst also overcoming taste and quality challenges sometimes associated with freshwater fish in the marketplace. In addition, the majority of tilapia producers have to use land based systems for the production of their fish, adding to their operational costs, which impact on their final production cost.

A number of important findings emerge. First, formal retail channels are not the most financially rewarding channels presently available to tilapia producers. Second, direct to market channels, catering for informal markets are financially rewarding for farmers. Third, markets show variances based on demographic composition. Fourth, new markets that trade on freshness as a prime differentiator are emerging. Fifth, consumer education is an important element in success.

These results are important to farmers and to policymakers as it provides them with insights into how early stage sustainability of farming operations can be achieved. This is particularly important to South African farmers as the sector is reaching a critical inflection point with respect to its continued growth and prosperity.

## TRANSCRIPTIONAL MODULATION AND MOLECULAR CHARACTERIZATION OF CATENIN $\beta$ FROM ROCK BREAM (*Oplegnathus fasciatus*)

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Catenin  $\beta$  is interesting protein as it plays a dual role in the cell, regulating the coordination of gene transcription and cell-cell adhesion. Firstly, cadherin receptor bind with intracellular cytoplasmic tail domains, it can act as an integral component of a protein complex in adherent junctions that helps cells maintain epithelial layers. Secondly, catenin  $\beta$  participates in the Wnt signaling pathway as a downstream target. The Wnt signaling pathways are a group of signal transduction pathways made of proteins that pass signals from outside of a cell through cell surface receptors to the inside of the cell. Anyhow molecular characterization of catenin  $\beta$  is limited in the fish. Therefore, study of rock bream catenin  $\beta$  isoform 1 (*RbCat $\beta$ 1*) is important for understand the expression and regulatory mechanism in fish. The Full length *RbCat $\beta$ 1* cDNA (2566 bp) comprised an open reading frame (ORF) of 2304 bp which encode a protein of 768 aa possessing molecular mass of 84 kDa and isoelectric point 5.3. Moreover, several conserved regions were identified with the comparison of other known orthologous such as; 12 ARM repeats, 6  $\beta$  catenin like repeats, HEAT domain and 11 protein binding sites. Phylogenetic analysis revealed that *RbCat $\beta$ 1* is closely clustered with *Maylandia zebra* (Zebra mbuna). Furthermore, pairwise comparison analysis was shown higher identities (98.8%) and similarities (99.3%) of *RbCat $\beta$ 1* with *Maylandia zebra*. Moreover, transcriptional studies revealed that *RbCat $\beta$ 1* was predominantly expressed in brain while moderately expressed in heart and liver tissues. Transcriptional profiling upon bacterial and viral inductions was shown up-regulation upon *E. tarda*, *S. iniae*, LPS, Poly I:C and rock bream iridovirus in liver tissue. Specifically, *RbCat $\beta$ 1* gene expression was higher ( $P < 0.05$ ) at 6 h p.i. of *E. tarda*, 48 h p.i. of LPS and *S. iniae* as a response of bacterial challenge. Furthermore, viral challenge of Poly I:C and rock bream iridovirus were shown significantly up-regulated *RbCat $\beta$ 1* gene expression at 48 h p.i. As a result post challenge results in liver tissue upon *E. tarda*, *S. iniae*, LPS, Poly I:C and rock bream iridovirus suggesting an immune role of catenin  $\beta$  1 in rock bream fish.

## COMMERCIALISATION OF SANDFISH AQUACULTURE IN THE KINGDOM OF SAUDI ARABIA

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The National Aquaculture Group (NAQUA) is located on the west coast of Saudi Arabia at 20°31'88.11"N, 40°04'58.22"E drawing water from the Red Sea. NAQUA is one of the most advanced, fully integrated, multi-functional marine aquaculture farms in the world, producing 18 000 tonnes of shrimp and 12 000 tonnes of Barramundi. The sandfish, *Holothuria scabra*, was identified as a candidate sea cucumber species for commercialisation and intensive production in shrimp ponds. The project started in 2010 with the initial focus on broodstock collection and the development of hatchery technology, spawning procedures and juvenile production. In 2013, NAQUA produced five million juveniles that were used in grow-out trials that aimed to optimise the farming life cycle. Since 2015, NAQUA has been spawning *H. scabra* consistently for 10 months of the year by thermal shock induction. The survival rate during settlement, from larvae to 0.2g juveniles, remains the main bottleneck to production. The survival rate was improved from 2.7% in 2013 to 3.8% in 2016, however this area has been identified for further research and development in 2017. Other technological advances over the past two years have focused on the development of a range of nursery systems to improve pre-grow-out survival rates (from 0.2g to g) which include happas (60 %), lined ponds (75%) and fiberglass trays (85%). Grow-out of *H. scabra* in a range of production systems, including water distribution channels and 10-hectare prawn ponds is demonstrated, with intensive technologies yielding an average biomass density of 600g m<sup>-2</sup>. During 2016, NAQUA harvested 60 000 kg of *H. scabra* with an average size of 350 g. This production was used to develop sales and marketing channels and optimise processing techniques to produce an A-grade sea cucumber product with an average processing yield of 8.7%. In 2017, the project will be fully commercialised, with a targeted production of 600 tonnes with a yield of 6 tonnes per hectare over a 12 month farming cycle.

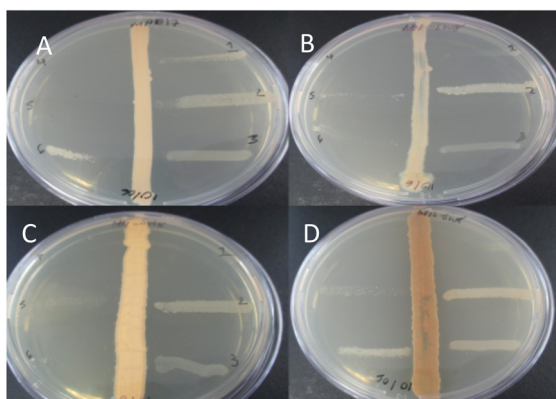
## ANTIMICROBIAL AND ANTI-BIOFILM POTENTIAL OF SEAWEED-ASSOCIATED BACTERIA ISOLATED FROM SOUTH AFRICAN SEAWEED AGAINST RESISTANT AQUACULTURE PATHOGENS

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The use of antimicrobial agents in aquaculture has significantly reduced options for treating fish diseases, due to the emergence of antimicrobial resistant-fish and opportunistic human pathogens. Research is now focused on the discovery of novel bioactive compounds, with seaweed being a prime resource in the search for microorganisms which demonstrate novel bioactivities. The antimicrobial and anti-biofilm potential of (n=96) seaweed-associated-bacteria (SWAB) from eight South African seaweeds, was thus investigated.

Isolates were tested for their antimicrobial activity utilizing primary screening (cross streak assay) following shake flask fermentation and ethyl acetate extraction. Secondary screening (agar well diffusion assay) against six bacterial fish pathogens and the minimum inhibitory concentration were determined. Extracts capable of inhibiting initial adhesion and mature biofilm were identified using concentrations ranging from 1-10 mg/ml utilizing crystal violet microtiter plate assay. Primary screening indicated that 67% of isolates displayed activity against *Aeromonas salmonicida*, 51% against *Edwardsiella tarda*, 16% against *Vibrio parahaemolyticus*, 12% against *Salmonella enterica serivar*, 11% against *Yersinia ruckeri* and 4% against *Aeromonas hydrophila*. Extract MAB24-SW1 (*B. velezensis*) demonstrated antibacterial effect against all aquaculture indicator strains. Extracts were most effective in the dispersal of mature biofilms with the best results being observed against *Aeromonas hydrophila* and *Vibrio parahaemolyticus*. The total range of biofilm inhibition by extracts was between 0.08% and 113.84%. Inhibition of initial adhesion was observed with 70% of extracts with pronounced activity observed against *V. parahaemolyticus* in a dose-dependent manner with limited effect on growth. Seaweed-associated bacteria could be used as a potential source for the isolation of bioactive metabolites to combat biofilm production and the associated antimicrobial resistance of aquaculture pathogens.



**Figure 1.1:** Primary screening results of (A) MAB17-SW1, (B) AB8-SW8, (C) AB9-SW8 and (D) AB12-SW8 against (1) *E. tarda* ATCC 15947, (2) *A. hydrophila* ATCC 7966, (3) *S. enterica* Arizona ATCC 13314, (4) *A. salmonicida* ATCC 33658, (5) *Y. ruckeri* ATCC 29473 and (6) *V. parahaemolyticus* ATCC 17802 aquaculture indicator bacteria using cross streak method. The antagonistic activity was indicated by inhibition of growth away from the vertically streaked indicator.



## IS RURAL SMALL-SCALE AQUACULTURE REALISED IN LIMPOPO PROVINCE, SOUTH AFRICA?

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ORAL PRESENTATION  
Theme: Pond Culture

### Introduction

The majority of rural households in South Africa are living under severe poverty. The availability of water varies considerably by region but even in areas with limited or erratic water supplies, maximising water use can increase agricultural productivity especially in the rural areas that are home to three quarters of the world's hungry people. Limpopo Province is endowed with resources that can play a vital role in developing small scale aquaculture development.

### Study Objective

The objective of the study was to develop sound standard small-scale aquaculture production system for rural communities of Limpopo Province.

### Methodology

The study was conducted in 2015 at the Elias Motsoaledi Municipality in Sekhukhune District. An existing small holding water earthen dam of the size of 56 000m<sup>2</sup> was divided into two portions. One portion of 23 000m<sup>2</sup> was developed into water storage dam for irrigation covered by HDPE lining. The other portion of 33 000 m<sup>2</sup> was developed and divided into six fish ponds of various sizes of 8346m<sup>2</sup>, 4662m<sup>2</sup>, 4464m<sup>2</sup>, 5040m<sup>2</sup>, 4160m<sup>2</sup> and 6391m<sup>2</sup> respectively. Ponds were lined with bentonite and crusher run to protect the walls.

### Results

The results showed that the development of standard rural aquaculture model has produced positive outcomes for rural small scale aquaculture farmers.

## **REVIEW OF RECENT DEVELOPMENTS IN AQUACULTURE IN CENTRAL AND SOUTHERN AFRICA, WITH PARTICULAR FOCUS ON THE ZAMBEZI RIVER - ZAMBIA / ZIMBABWE**

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Aquaculture has recently received renewed attention in Central Africa.

This paper assesses historic and current development of aquaculture in Zambia and Zimbabwe, with more information from the region in general. Various changes to the political and economic environment over the last decade are examined, and tentative predictions for future growth are discussed.

Up to date information on the rapidly changing situation in Zambia will be reviewed, and the locally developed technology to extend this form of economic activity through the Aquaculture Development Association of Zambia to the many small scale pond farmers struggling with low yields is elaborated.

## EVALUATION OF THE USE OF A PROTOCOL OF SEMINAL CRIOPRESERVATION IN TELEOST BRAZILIAN

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Common Curimatã (*Prochilodus brevis*) and Tambaqui (*Colossoma macropomum*) are two rheophilic species of teleosts, from the same order (Characiformes) found in South America and of great economic and ecological importance. However, due to the anthropic actions, the reproduction of these species has been threatened. Seminal cryopreservation is one of the alternatives to minimize this problem; however, it is observed in the literature that the protocols of seminal cryopreservation may differ between species. Thus, before and after this technique, kinetic and morphological analyzes are carried out, among others, to evaluate the seminal quality. Therefore, this work aimed to evaluate if the seminal cryopreservation protocol confers the same seminal quality after thawing in these two teleost species.

Males ( $n = 8$ ) of each species were hormonally induced with pituitary carp extract ( $3 \text{ mg kg}^{-1}$  to common curimatã and  $2.5 \text{ mg kg}^{-1}$  to tambaqui) to induce spermiation. After 18 h of induction, the animals were anesthetized and semen was collected individually. After collection, the motility of the semen was evaluated with the aid of Sperm Class Analyzer (SCA) and other aliquot fixed in formalin citrate solution for morphology. For cryopreservation, the semen samples were diluted in (1) glucose + DMSO-dimethyl sulfoxide and (2) glucose + MG-methyl glycol, packaged into 0.25 mL straws and put into a programmed freezing machine. After 10 days, the semen samples were thawed and evaluated with regard to kinetics and sperm morphology with the aid of the SCA software. For a statistical analysis, the data were submitted to one Way ANOVA followed by Tukey's test ( $p < 0.05$ ) with the aid of ASSISTAT™ software version 7.7 beta (2016) and presented as mean  $\pm$  standard deviation ( $M \pm SD$ ).

The cryopreservation treatments showed different results between species (Table 1). The treatment Glucose + DMSO conferred better motility (%) and normal sperm (%) compared to Glucose + MG in *C. macropomum*, and the opposite was observed in *P. brevis*.

Therefore, the results indicate that a single seminal cryopreservation protocol does not guarantee the same results in all freshwater fish species, therefore it is species-specific, and adaptations and changes of diluting solutions are necessary for the elaboration of an adequate protocol for seminal cryopreservation.

TABLE 1. Sperm motility and normal spermatozoa (%) of *Colossoma macropomum* and *Prochilodus brevis* ( $M \pm SD$ ). Different letters in the same column represent differences between treatments ( $p < 0.05$ ).

|                | <i>Colossoma macropomum</i> |                   | <i>Prochilodus brevis</i> |                  |
|----------------|-----------------------------|-------------------|---------------------------|------------------|
|                | Motility (%)                | Normal (%)        | Motility (%)              | Normal (%)       |
| Glucose + DMSO | $23.8 \pm 4.3^a$            | $51.4 \pm 13.9^a$ | $20.5 \pm 7.4^b$          | $62.7 \pm 7.2^b$ |
| Glucose + MG   | $6.0 \pm 2.3^b$             | $24.9 \pm 3.0^b$  | $71.4 \pm 6.2^a$          | $75.4 \pm 1.7^a$ |

Acknowledgement: FAPESP (grant #2014/16320-7; grant #2016/08770-8).

## NUTRITIONAL MODELLING OF PROTEIN, AMINO ACID AND ENERGY REQUIREMENTS OF TIGER GROUPER *Epinephelus fuscoguttatus*

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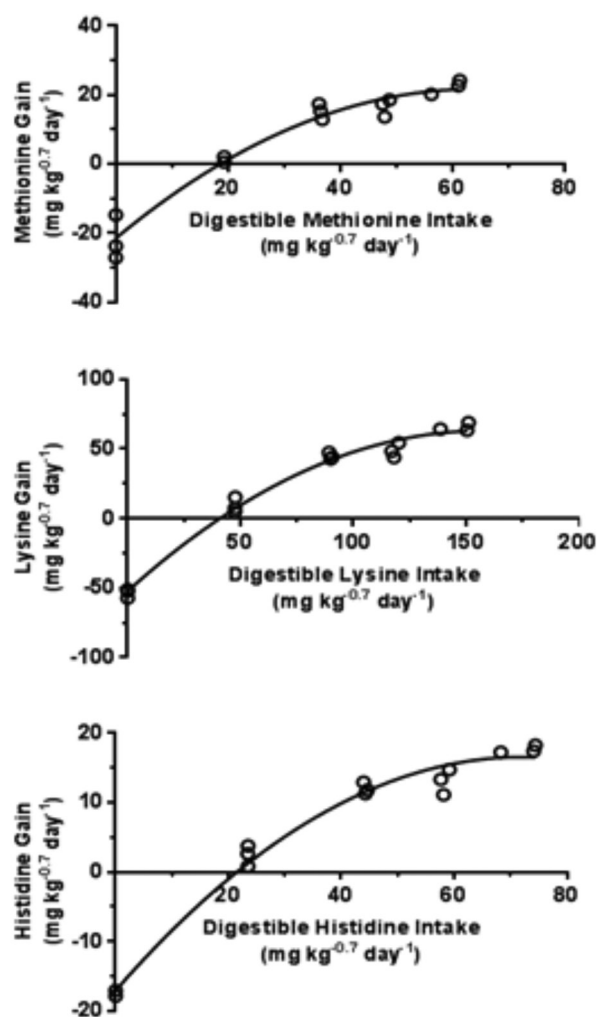
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Tiger grouper are a high value marine fish species yet relatively little information is available describing their basic nutritional requirements for protein and energy. Nutritional modelling has been successfully applied to many aquaculture species to provide quantitative estimates of nutrient and energy requirements throughout growth production. While models have been developed for several species describing protein and energy requirements, few have considered the requirements for essential amino acids.

This study used a bioenergetic approach to quantify the digestible protein, amino acid and energy utilisation efficiencies, maintenance requirements, carcass composition and growth potential of tiger grouper. The daily digestible protein (DP) intake to achieve maximum predicted protein retention efficiency was  $2.0 \text{ g DP kg}^{-0.7} \text{ day}^{-1}$ . The utilization efficiency of dietary protein for tiger grouper was 0.58 with the corresponding cost of DP per unit of protein gain was  $1.71 \text{ g g}^{-1}$ . The daily digestible energy (DE) intake ( $\text{kJ DE kg}^{-0.8} \text{ day}^{-1}$ ) to achieve a maximum predicted energy retention efficiency was  $101 \text{ kJ DE kg}^{-0.8} \text{ day}^{-1}$ . The utilization efficiency of energy for tiger grouper was 0.63 and equates to an energetic cost of production of  $1.59 \text{ kJ DE kJ}^{-1}$  energy deposition. Utilisation efficiencies for digestible MET, LYS and HIS were 0.51, 0.54 and 0.30 respectively (Fig. 1). The reciprocal cost of production for these essential amino acids per unit gain was 2.0, 1.8 and  $3.4 \text{ g g}^{-1}$  respectively.

Detailed understanding of nutrient and energy requirements of tiger grouper throughout production will provide a platform to improve feed management and feed formulation.



**Fig. 1.** Relationship between digestible amino acid intake ( $\text{mg kg}^{-0.7} \text{ day}^{-1}$ ) and amino acid gain ( $\text{mg kg}^{-0.7} \text{ day}^{-1}$ ) in tiger grouper.

# THE CRITICAL OXYGEN THRESHOLD OF YELLOWTAIL KINGFISH *Seriola lalandi* ACCLIMATED TO 15°C AND 20°C

Igor Pirozzi<sup>\*1,2</sup>, Caroline Candebat, Mark Booth

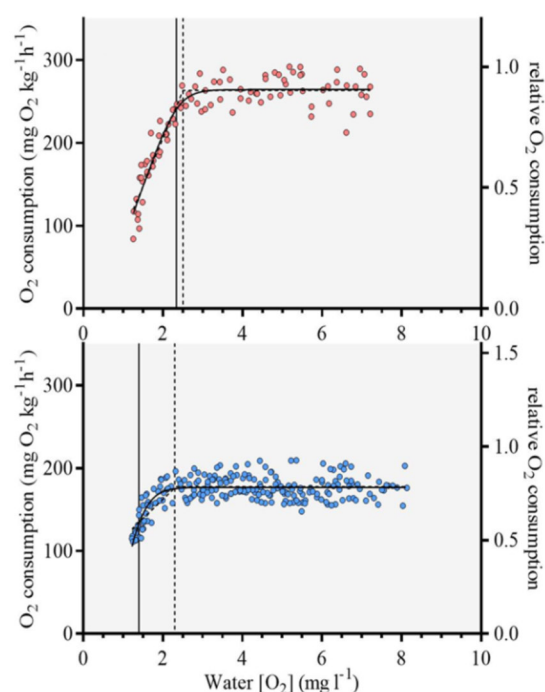
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Dissolved oxygen is one of the first limiting abiotic factors in aquaculture systems potentially impacting on the health and wellbeing of aquatic animals. The commercial aquaculture of Yellowtail kingfish (YTK) is rapidly expanding globally yet the critical dissolved oxygen limits of YTK are currently unknown. This study quantified the critical oxygen level ( $P_{crit}$ ) of YTK at two temperature (15°C & 20°C).

YTK initially weighing approximately 250 g were stocked into two separate 10kL tank recirculating aquaculture systems at ambient temperature (~18°C) and the temperature then adjusted over a period of five days until the respective experiment temperatures were reached. YTK were then held for a further 45 days. Over this period fish were fed a 6mm feed with dietary specification of 56% crude protein and 25 MJ/kg energy. Following the acclimation phase four fish were each placed into 200L respirometry chambers ( $n=3$ ). Fish were given time (~45 min) to settle in the respirometers before water flow and aeration were removed and chambers sealed to create a static environment. This habituation phase was considered sufficient as evidenced by a consistent routine metabolism (horizontal phase; Fig. 1). Oxygen consumption was measured using Pyroscience FireStingO2 units.  $P_{crit}$  was established in YTK as the critical threshold of oxygen regulation to oxygen conformation indicated by a continued decline in respiration rate. This value was statistically quantified using both non-linear multiphasic and segmented linear regression analyses. Estimates of  $P_{crit}$  between models were significantly different in cool water but not in warm water. Segmented linear analyses gave the most conservative estimate of  $P_{crit}$ . Hypoxia tolerance in YTK is dependent on the acclimation temperature with YTK acclimated to 20°C ( $P_{crit} = 2.5 \pm 0.1$  mg O<sub>2</sub> l<sup>-1</sup>) significantly less tolerant to hypoxia than fish acclimated to 15°C ( $P_{crit} = 2.3 \pm 0.7$  mg O<sub>2</sub> l<sup>-1</sup>) based on a segmented linear model.



**Fig. 1.** Respiration rate of YTK in closed cell respirometers at 20°C (top) and 15°C (bottom). Dashed vertical line =  $P_{crit}$  breakpoint estimated using segmented linear regression. Solid vertical line =  $P_{crit}$  breakpoint estimated using non-linear multiphasic regression.

## LOW DISSOLVED OXYGEN AFFECTS AMINO ACID UTILISATION AND MAINTENANCE REQUIREMENTS IN YELLOWTAIL KINGFISH *Seriola lalandi*

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This study investigated the effect of low dissolved oxygen saturation (60%) compared to normoxic saturation (100%) on the nutrient and energy utilisation of sub-adult Yellowtail kingfish (YTK). A six week growth experiment using juvenile YTK (220.9±2.35 g) was carried out in two separate recirculating aquaculture systems held at 20 °C. One system was allocated as low DO saturation (S60; Avg. DO 61.8% ±0.54 SE) and the other as normoxic DO (S100; Avg. DO 98.2% ±0.85 SE). Each experiment system consisted of a 10kL tank containing ten 200L cages with ten YTK randomly distributed into each duplicate cage per treatment. Depending on the DO treatment group the DO level was controlled either by N<sub>2</sub> gas injection and/or normal aeration or O<sub>2</sub> gas. YTK were fed a commercial 6mm diet one of five feeding levels (L1-L5) ranging from approximate maintenance ration (L1) to apparent satiation (L5). Digestibility of the diet was determined in each of the DO environments with the satiated group at the conclusion of the feed trial.

There was no significant difference in feed intake or crude nutrient and energy intake between each of the paired ration levels at either DO saturation. However, on a digestible basis, fat intake was significantly greater in 100S L5 fish while histidine intake was significantly greater in S60 L5 fish. This is reflected in the different ADCs of these nutrients at different oxygen environments. Digestible intake was similar for all other respective nutrients and dietary energy between 100S L5 and 60S L5 fish. The rate of amino acid deposition relative to digestible amino acid intake was significantly different between DO treatments for all essential amino acids except for threonine and phenylalanine (Fig. 1).

The average utilisation co-efficient value of all essential amino acids was 0.49 (*cf.* protein 0.41). Where there were significant differences between DO treatments amino acid utilisation efficiency decreased in low DO saturation. However, the influence of DO on maintenance requirements varied depending on the amino acid, for e.g. Histidine maintenance requirement = 26.0 and 20.7 mg kg<sup>-0.7</sup> day<sup>-1</sup> for S60 and S100 respectively while the maintenance requirement for arginine was 38.4 and 48.5 mg kg<sup>-0.7</sup> day<sup>-1</sup> for S60 and S100 respectively.

Low DO at 60% saturation negatively affected the nutrient and energy utilisation response in YTK with this response tending to be more pronounced with increasing nutrient and energy intake. Data generated from this study can be used to improve feed models for YTK facilitating better feed management and feed formulation through a better understanding of nutrient requirements and therefore dietary specifications for YTK.

## HARMFUL ALGAL BLOOMS AND THE SOUTH AFRICAN AQUACULTURE SECTOR: YESSOTOXINS AND THEIR IMPACTS

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In December 1966, the death of hundreds of thousands of white mussels on the west coast of South Africa was associated with a bloom of an unknown dinoflagellate species, later described as *Gonyaulax grindleyi*. It is likely that this mortality is the first recorded impact of yessotoxins (YTXs) on the South African coast, although the toxic properties of *G. grindleyi* (later renamed *Protoceratium reticulatum*) and its production of YTXs was only confirmed in New Zealand in 1997. In 2005, the first major impact of YTXs on the aquaculture sector in South Africa followed a bloom of *P. reticulatum* in Saldanha Bay. YTX concentrations in mussels ranged from 1.02 – 3.6 mg kg<sup>-1</sup> and were the cause of a 5 month closure of mussel farming in the bay. Subsequent application in South Africa of less stringent regulatory limits for YTX in bivalves reduced the risk of closure of shellfish operations.

Studies in the Adriatic Sea in 1999 and in New Zealand in 2006 identified two other YTX-producing dinoflagellates *Lingulodinium polyedrum* and *Gonyaulax spinifera*. In 2014, an exceptional bloom of *L. polyedrum* was observed for the first time on the coast of South Africa. Persisting for several months on the south coast, this bloom is considered to have impacted negatively on sardine catches. Following this unprecedented event the south coast was again subject to an extensive bloom in 2017. Dominated by both *G. spinifera* and *L. polyedrum*, this bloom was the cause of massive stock losses on abalone farms in the Walker Bay area. YTXs dominated by the analogue homo-YTX are considered to have disrupted cell adhesion, causing a loss of functional gill epithelium particularly in younger abalone and in aging brood stock. The loss to abalone farmers, valued at several million US\$, represents the largest single loss to the aquaculture sector of South Africa.



## PROSPECTIVE FOR AQUACULTURE IN THE FACE OF GLOBAL CLIMATE CHANGE IN VERACRUZ, MEXICO

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Global Climate Change (GCC) will have profound implications for capture fisheries and aquaculture. According to projections by the Intergovernmental Panel on Climate Change (IPCC), decline in capture fisheries of up to 40 % is expected by 2025 in tropical and subtropical seas. Mainly because of increments in sea surface temperature and acidification of oceans by increased carbon dioxide emissions. In the other hand, Aquaculture will be negatively impacted mainly by increased temperature, increased frequencies of tropical storms and hurricanes and, ocean acidification. However, as this activity can be carried out in controlled environments, it is considered by FAO as the only option in order to keep up with world seafood demand.

Veracruz State is located in the Mexican east coast, and is subject to frequent tropical storms and hurricanes. It is considered as one of the most vulnerable States to climate change effects and, it is one of the main tilapia producing states in Mexico with around 800 tilapia farms, mostly small farms, self-consumption farms and some commercial farms. This research was carry out surveying 800 fish farm and applying a questionnaire and localized the farms by GPS. With this information we analyzed the vulnerability of producers to GCC. The aim of the present study was to discuss possible alternatives and suggested solutions to cope with detected negative impacts of climate change on aquaculture in Veracruz State in order to increase tilapia aquaculture production to the required level to satisfy seafood demand.

Past experience with hurricane Karl in 2010 have taught that farms below 15 meters above sea level (MASL) were flooded. With this information and sea level of individual farms, it is estimated that around 24 % of farms and 40 % of tilapia state production is under flood risk during category 3 hurricanes. Additionally strong winds can destroy aquaculture infrastructure such as geomembrane fish tanks, electric installation, wooden warehouses, etc. Rising temperatures can affect tilapia aquaculture in different ways but mainly during extremely hot days, water temperature in tanks can rise above 36°C and last for up to 15 days, causing decreases in water oxygen content and in food consumption, leading to decreased growth and outbreaks of diseases like hemorrhagic septicemia and exophthalmia causing fish mortality. Currently, during warmer months around 12% farms and 16% of production are affected by water temperatures above 36°C, some farmers' anecdotal information indicate that this situation can be controlled with the use of probiotics. It is estimated by projections that in short term to 2039 around 28 % tilapia production will be affected. Meanwhile, long term projection estimate that 91% of production will be affected.

It is suggested that to ameliorate the detected negative effects of climate change, it is required that site selection for aquaculture farms must include an altitude above 20 MASL. Careful selection on construction materials for tanks and warehouses such as concrete to stand strong wind forces. It was detected the need for onsite research for the control of disease outbreaks using probiotics as viable disease control measure during the warmer months. Furthermore, it is discussed the need for a national aquaculture policy considering climate change implications.

## SKIN ULCERS IN COD *Gadus morhua* FROM THE SOUTHERN BALTIC SEA

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Ulcer syndrome is one of the most common diseases affecting cod *Gadus morhua* from the Baltic Sea. The development of skin ulcers may be associated with water pollution, environmental changes, infectious agents and other factors. An investigation of externally visible symptoms of skin lesions in cod was conducted during research surveys in the southern Baltic in April 2016. Fish with ulcers were subjected for further bacteriological examination by classical and molecular methods. Generalized linear models (GLM) were applied to analyse the prevalence of ulcers in cod relative to biological and spatial parameters. Significant, negative correlation was found between the presence of ulcers and Fulton's body condition factor of cod. The highest prevalence of ulcers was reported in the Gulf of Gdansk which is regarded as one of the most polluted areas in the entire Baltic Sea, classified as disturbed by hazardous substances. Bacteriologically, *Pseudomonas* spp., *Aeromonas* spp., *Shewanella* spp., *Chryseobacterium* spp. were mostly isolated from skin ulcers of fish. The presence of toxic pollutants in the marine environment may increase susceptibility of fish to bacterial infections. This research was supported by The National Centre for Research and Development under the Strategic Program Biostrateg II (grant no. 296211/4/NCBR/2016).

Samples of cod were collected in the following areas: BS - Bornholm S; BN - Bornholm N; KD - Kolobrzeg-Darłowo; SF - Slupsk Furrow; GG -Gulf of Gdansk. A total number of 667 individual fish were examined. Total length of fish (cm), body mass (g), sex, and gonad developmental stage (according to Maier's scale) were recorded. Fulton's formula was used to determine the body condition factor, FCF:

$$FCF = w/l^3 \times 100$$

where w - total weight and l - length of fish.

Ninety-eight diseased fish were collected for bacteriological examination from which more than 500 bacterial strains were isolated.

GLM analysis was performed in GenStat statistical package. Error distribution was assumed to be binomial and the logit link function was used. Mean prevalence of ulcers in cod and the effect of sampling area are presented in Figure 1.

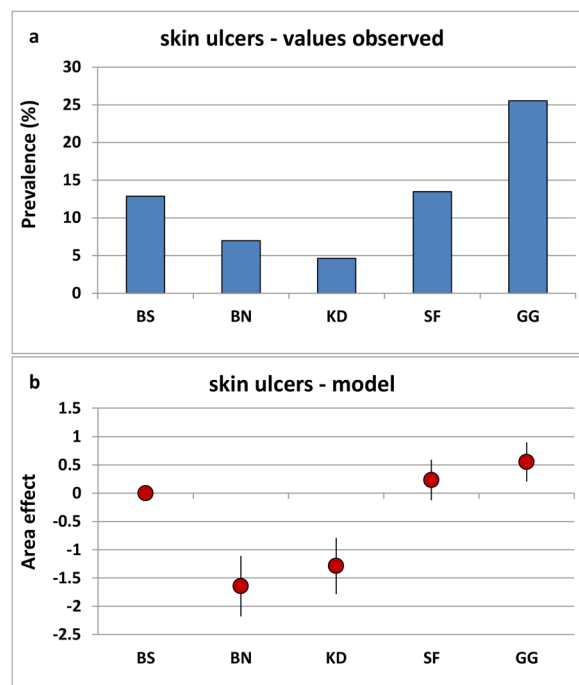


Figure 1. The prevalence of skin ulcers in cod: a - values observed and b - relative to the area effect included in the model (with standard errors).

## NILE TILAPIA NURSERY IN AQUAPONIC SYSTEM USING BIOFLOC TECHNOLOGY: PRELIMINARY RESULTS<sup>1</sup>

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Aquaponics is an environmentally friendly system, contributing to water saving, nutrient recycling, land conservation, and production of high quality fish and vegetables. Consequently, aquaponics emerges as an alternative for aquaculture diversification. Additionally, aquaponics may be more efficient in the presence of a diverse microbial community, such as in biofloc technology (BFT). The purpose of this research was to evaluate the efficiency of BFT on the aquaponic production of Nile tilapia *Oreochromis niloticus* juveniles and smooth lettuce (*Lactuca sativa* L.).

During 46 days we studied the production of one cycle of fish and two cycles of lettuce (23 days each), and two treatments were evaluated: BFT and CW (clear water - control), with three replicates each. Each unit (replicate) consisted of one tank of 500L for tilapia culture (~ 1g initial weight, 300 fish.m<sup>-3</sup>) and three floating beds for hydroponic culture in BFT or CW. Additionally, the CW had a filtration system with two mechanical and one biological filters, while in the BFT only clarifiers were used to minimize the accumulation of flakes on the roots of plants.

The results suggested that BFT provided better plant growth only in the first production cycle, as in the second cycle the opposite occurred (better in CW). Several biological and engineering factors may have influenced the relationship between decreased plant productive performance and maturation of the biofloc system. The plant productivity (kg.m<sup>-2</sup>) in the first and second cycles on the BFT system was respectively 2.96 and 1.80 kg.m<sup>-2</sup>, while on the AC was 2.61 and 2.62 kg.m<sup>-2</sup>. On the other hand, fish performance in BFT was better as compared to CW, except the productivity and survival with no significant differences (P>0.05). The results suggest that BFT will be an alternative for the aquaponic production only if more studies are carried out to understand the factors that negatively affected plant growth in matured systems.

Table 1 - Mean ( $\pm$  standard deviation) of productive performance of Nile tilapia juvenile in aquaponics system, during the experimental period (46 days).

| Zootechnical Parameters            | CW                  | BFT                | Value of P* | CV (%) |
|------------------------------------|---------------------|--------------------|-------------|--------|
| Final weight (g)                   | 34.85 $\pm$ 0.41 b  | 36.69 $\pm$ 0.6 a  | 0.012       | 1.45   |
| Total length (cm)                  | 12.15 $\pm$ 0.041 b | 12.42 $\pm$ 0.11 a | 0.016       | 0.66   |
| Productivity (kg m <sup>-3</sup> ) | 7.85 $\pm$ 0.11 a   | 8.05 $\pm$ 0.12 a  | NS          | 1.45   |
| SGR (% day <sup>-1</sup> )         | 7.43 $\pm$ 0.04 b   | 7.55 $\pm$ 0.05 a  | 0.023       | 0.58   |
| FCR                                | 0.88 $\pm$ 0.01 b   | 0.85 $\pm$ 0.01 a  | 0.037       | 1.49   |
| Survival (%)                       | 99.00 $\pm$ 1.33 a  | 96.47 $\pm$ 1.78 a | NS          | 6.92   |

\* Lowercase letters indicate differences between the production systems (5% significance)

## DETERMINATION OF EXTERNAL MORPHOLOGICAL MARKERS TO EVALUATE THE OPTIMAL TIMING FOR REPLACEMENT OF LIVE BY FORMULATED FOOD IN PACU (*Piaractus mesopotamicus*) LARVAE: PRELIMINARY RESULTS

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The production of pacu larvae and juvenile is still challenging as it involves the development of hatchery protocols to ensure production of high quality juveniles at a reasonable price. The pacu larvae depend on live food for normal early development. Therefore, the characterization of the external development of the pacu larvae during the initial phase and its correlation with the organogenesis of the digestive tract can guide the producers and the production routine by determining the best time to start the weaning. The present study aimed to characterize external morphological markers correlating the organogenesis of the digestive tract in pacu larvae (*Piaractus mesopotamicus*) fed with different feeding strategies. After mouth opening (3<sup>rd</sup> to 4<sup>th</sup> day post-hatching, dph), larvae were distributed into 15 100-L tanks (10 larvae.liter<sup>-1</sup>). The experiment was conducted during 30 days after first feeding, in randomized design, consisting of three treatments and five replicates: early weaning (WP), late weaning (WT) and a control with only live feed (C). Notochord flexion, the development of paired and unpaired fins, the morphometric and meristic characteristics, the pattern of pigmentation and appearance of scales were observed. The first pacu larvae sample was collected at 5 dph. Until that day, they were at yolk-sac larvae phase. The pre-flexion phase began at 6 dph being possible to observe the end of the notochord still stretched. The flexion phase started approximately at 12<sup>th</sup> dph when the beginning of notochord flexion was observed in all treatments. The larvae also displayed chromatophores containing dark pigments in the ventral part of the body, more intense in the posterior part of the head and trunk. Still, we could see the beginning of the formation of the dorsal and caudal fins. At 14 dph, the flexion of the notochord was more advanced in C and WT treatments, and identification of hypural bone was possible. At this stage, the larvae exhibited dorsal and caudal fins well developed and starting the reddish pigmentation. At 16 dpe, we observed the complete formation of the dorsal, caudal and anal fins and hypural bone, characterizing the post-flexion stage. Until 21 dph we observed the early development of the pelvic fins, more evident in larvae from WT and WP treatments, as well as yellow and red pigmentation in the fins. To date, histological analysis are in progress, but the partial results of morphometric and meristic characteristics, flexion of the notochord and pigmentation pattern already provide the first morphological indicators of pacu larvae to be correlated in the future with the development of the digestive tract.

**Table 1.** Main morphometric measurements (mm) of larvae in each treatment throughout the experiment (dph).

| dph | Cp  |     |      |      | A   |     |     |     | Cc |     |     |     |
|-----|-----|-----|------|------|-----|-----|-----|-----|----|-----|-----|-----|
|     | 5   | 10  | 16   | 22   | 5   | 10  | 16  | 22  | 5  | 10  | 16  | 22  |
| C   | 3,8 | 5,3 | 9,2  | 11,2 | 0,3 | 0,4 | 1,3 | 2,0 | -  | 1,3 | 2,5 | 3,5 |
| WP  | 3,9 | 5,8 | 9,8  | 12,4 | 0,3 | 0,4 | 1,2 | 2,3 | -  | 1,1 | 2,7 | 3,9 |
| WT  | 3,8 | 4,9 | 10,2 | 12,0 | 0,3 | 0,4 | 1,4 | 2,2 | -  | 1,3 | 3,0 | 3,7 |

Cp: standard length, A: body height, Cc: bone head length;

**Table 2.** Number of fin rays in pacu larvae in each treatment throughout the experiment (dph).

| dph | Df |    |    |    | Cf |    |    |    | Af |    |    |    |
|-----|----|----|----|----|----|----|----|----|----|----|----|----|
|     | 5  | 10 | 16 | 22 | 5  | 10 | 16 | 22 | 5  | 10 | 16 | 22 |
| C   | -  | -  | 14 | 15 | -  | -  | 20 | 21 | -  | -  | 21 | 24 |
| WP  | -  | -  | 15 | 14 | -  | -  | 20 | 20 | -  | -  | 21 | 24 |
| WT  | -  | -  | 15 | 15 | -  | -  | 20 | 21 | -  | -  | 22 | 25 |

Df: dorsal fin, Cf: caudal fin, Af: anal fin.

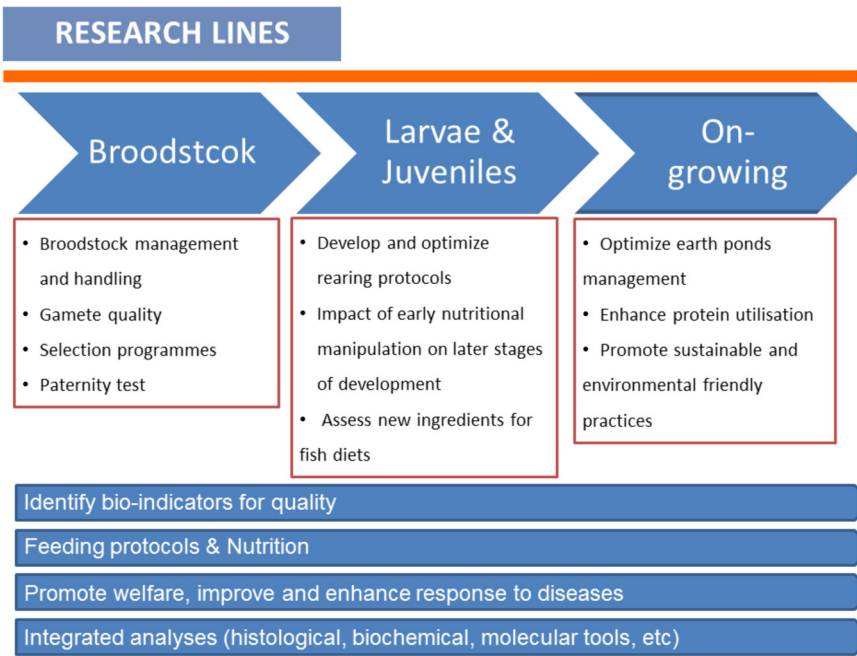
**AQUACULTURE RESEARCH IN SOUTHERN EUROPE: TRADITIONAL AND EMERGING MARINE FISH SPECIES**

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Europe needs to highly increase their aquaculture production for different marine species in order to supply consumer needs and diversify products. Still, this activity has to increase facing challenging European regulations on animal welfare, respect environment and obtain high quality products. To attain this ambitious objective is important to implement research strategies integrated with the industry. IPMA’s aquaculture research station, in the South of Portugal, is a marine core facility with conditions to perform small and large scale research.

This communication aims to present the research on traditional and emerging fish species, perspectives and production potential in the Southern Europe. The large experience achieved with the research on developing and optimizing rearing protocols for gilthead and white seabream, sole, sea bass and meagre provide the skills to assay species with high potential for aquaculture, like fast growing species (eg. amberjack, mahi-mahi) or important species whose wild stocks are decreasing (eg. sardine). These species can be reared in RAS system that can be integrated with clean and competitive source of energy (eg. photovoltaic). Moreover, these species have potential for processing (eg. fillet, sushi, canning, etc. )



**Acknowledgments:** Research funded by project DIVERSIAQUA (MAR2020, Portugal)

## NEW SPECIES BUT WITH SAME SYSTEMS? THE CASE OF MEAGRE IN SOUTHERN EUROPE

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Europe needs to highly increase their aquaculture production for different marine fish species in order to supply consumer needs and diversify products. New species with fast growth rates and high potential for processing may help achieve this. At IPMA's Aquaculture Research Station, meagre (*Argyrosomus regius*) has been evaluated in all its cycle and the growout has been mainly carried out under earth pond conditions, commonly used for seabream and seabass.

Monoculture and polyculture combinations has been tested with seabream, seabass and in later years also with oysters under IMTA practices. Compared with these fish species, meagre as shown a higher growth rate in summer months but lower during winter (Fig. 1), being almost null below 12 °C, common temperature in most of the coastal aquaculture farms in Portugal. Trials at EPPO with heated water during winter has given some insights that meagre can maintain higher growth rates thus reducing production cycle. These results highlight the potential of this species to be cultured under Recirculating Aquaculture Systems with temperature control above 20°C.

In earth ponds meagre growth was not affected when in combination with oysters but higher total biomass was obtained when this combination was used (Fig. 2). Also preliminary results on water quality showed that oysters may control microalgae blooms and oxygen fluctuations and also turbidity levels, giving better visualization of fish.

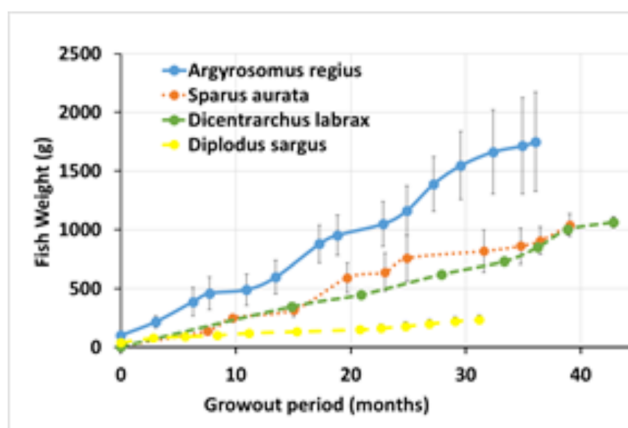


Figure 1 – Meagre growth compared with other species in EPPO earth ponds

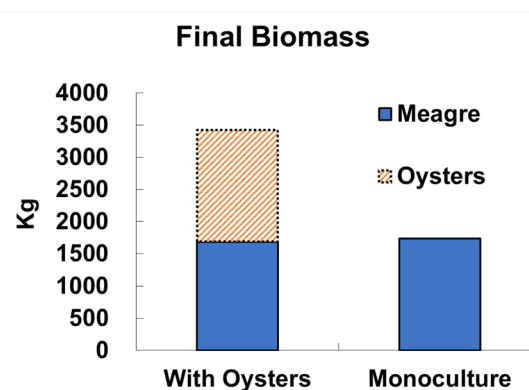


Figure 2 – Final biomass production at EPPO when meagre is reared with oyster or in monoculture

**Acknowledgements:** Research funded by project DIVERSIAQUA (MAR2020, Portugal).



## NUTRIENT DIGESTIBILITY OF SELECTED MICROALGAE FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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The global production of fisheries and aquaculture sector will reach 172 million tons in 2021 and this raise will be attributable mostly to aquaculture. Fishmeal is currently the main source of protein utilized to feed for Pacific white shrimp, *Litopenaeus vannamei*; however, the overexploitation of fisheries resources combined with the growing demand has caused that the price of fishmeal is continuously rising and unsustainable. Many studies have been focused on the search of alternative protein sources, which might reduce the dependency on fishmeal. Any alternative protein source should ideally meet some important aspects (high nutritive value, adequate amino acid and fatty acid profiles, secured supplies). Vegetable proteins contain a wide range of antinutrients (phytic acid, trypsin inhibitor, haemagglutinin, and antivitamin). High inclusion level of these ingredients can induce negative effects on growth and digestive enzyme activities. Microalgae contain a protein level ranging from 30 to 55% DM, about 13–33% DM total lipids; therefore, these ingredients might well reduce dependency of fishmeal. The aims of this study are: (1) to determine the nutritive values of selected microalgae (*Gracilaria fisheri*, *Scenedesmus dimorphus* KMITL strain and *Athrospira platensis*) and (2) to evaluate the digestibility of microalgae in the diet of *L. vannamei*. A 4-week feeding trial was conducted for the apparent digestibility study in a static water system with about 5.8 g shrimp<sup>-1</sup>. Each diet (Table 1) was fed ad libitum to three replicate groups of shrimp twice daily (08:30 a.m. and 16:30 p.m.). After feeding, unfed and residue were removed by siphoning. Fecal collection was done by siphon prior to the next meal during 28 consecutive days. We found that Spirulina had higher protein content (59.9%) than *Gracilaria* (12.9%) and *Scenedesmus* KMITL strain (15.1%). The apparent protein digestibility of *Gracilaria* and Spirulina for *L. vannamei* were 77.8 and 73.7%, respectively. *Scenedesmus* KMITL strain had highest lipid content (6.9%) and the apparent digestibility of lipid (66.6%) (Table 2).

**TABLE 1: Feed formulation of experimental diets fed to Pacific white shrimp (%)**

| Ingredient                     | Reference | <i>Gracilaria</i><br>( <i>G. fisheri</i> )* | <i>Scenedesmus</i><br>( <i>S. dimorphus</i> KMITL strain)* | <i>Spirulina</i><br>( <i>Athrospira platensis</i> )* |
|--------------------------------|-----------|---|--|--|
| Local fishmeal, protein 55     | 60.0      | 42.0  | 42.0   | 42.0   |
| Test ingredient*               | 0.0       | 30.0  | 30.0   | 30.0   |
| Fish solubles                  | 1.0       | 0.7   | 0.7  | 0.7  |
| Wheat flour                    | 30.0      | 21.0  | 21.0   | 21.0   |
| Fish oil                       | 3.0       | 2.1   | 2.1  | 2.1  |
| Monocalcium phosphate          | 2.0       | 1.4   | 1.4  | 1.4  |
| CaCO <sub>3</sub>              | 2.0       | 1.4   | 1.4  | 1.4  |
| Vitamin-mineral premix         | 2.0       | 1.4   | 1.4  | 1.4  |
| Cr <sub>2</sub> O <sub>3</sub> | 0.5       | 0.5   | 0.5  | 0.5  |

\* each test ingredient was included by 70:30 (reference: test ingredient)

**TABLE 2: Proximate compositions and apparent digestibility coefficients of selected microalgae (% as dry matter basis)**

|  | <i>Gracilaria</i><br>( <i>G. fisheri</i> ) | <i>Scenedesmus</i><br>( <i>S. dimorphus</i> KMITL strain) | <i>Spirulina</i><br>( <i>Athrospira platensis</i> ) |
|--|--|---|---|
| <i>Proximate composition</i>               |  |   |   |
| Dry matter                                 | 98.4                                       | 95.1  | 99.3  |
| Protein                                    | 12.9                                       | 15.1  | 59.9  |
| Lipid                                      | 3.6  | 6.9   | 2.7   |
| Ash  | 28.0                                       | 47.9  | 8.3   |
| Nitrogen free extract <sup>a</sup>         | 55.5                                       | 30.1  | 29.1  |
| <i>Apparent digestibility coefficients</i> |  |   |   |
| Protein                                    | 77.8                                       | 6.3   | 73.7  |
| Lipid                                      | 42.4                                       | 66.6  | 17.4  |
| Ash  | 112.3                                      | 88.5  | 126.9   |

<sup>a</sup> Nitrogen free extract (NFE) = 100 – (protein + lipid + ash)



# **MONOGENEAN PARASITES OF *Clarias gariepinus* FROM SOUTHERN AFRICA: BASELINE DATA TO IDENTIFY POTENTIAL PATHOGENS OF AQUACULTURAL IMPORTANCE**

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The Sharptooth catfish, *Clarias gariepinus* (Burchell), is one of the most widely distributed freshwater fishes in Africa. It has been introduced under either controlled or uncontrolled conditions to different regions of Africa, and are also found in Israel, Lebanon and Turkey. As a result of the ability to tolerate extreme environmental conditions, *C. gariepinus* is a popular aquaculture species and is highly recommended as a food source. As such, it has also become a popular candidate for research, including parasitological work on Monogenea. Some monogenean species are highly pathogenic and in aquaculture high infection rates may result in high mortality causing subsequent economical loss.

From August 2011 to September 2016, field surveys were undertaken at eight localities in South Africa and two in Zimbabwe, and included the Zambezi, Limpopo and Vaal river basins. A total of 43 specimens of *C. gariepinus* were collected and studied for the presence of monogenean parasites. Morphometrical and molecular characterisation of parasites were performed to confirm their identity.

Seventeen species from three genera were recorded as follows: four species of *Quadriacanthus* Paperna, 1961, three species of *Macrogyrodactylus* Malmberg, 1957 and 10 species of *Gyrodactylus* von Nordmann, 1832. The species composition varied among sampled sites, with the highest species diversity recorded from the locality inside of Kruger National Park.

All *Quadriacanthus* and *Macrogyrodactylus* species collected are known, but an unexpected six new *Gyrodactylus* species were sampled.

Results from this study, based on a single, widely distributed host, provides useful baseline information towards the identification of potential pathogens.

**TABLE 1. Monogeneans recorded from studied sites.**

| <b>South Africa</b> | NL | River Basin | NF | Parasite genus           | NS |
|---------------------|----|-------------|----|--------------------------|----|
| Limpopo Prov.       | 5  | Limpopo     | 23 | <i>Gyrodactylus</i>      | 8  |
|                     |    |             |    | <i>Macrogyrodactylus</i> | 3  |
|                     |    |             |    | <i>Quadriacanthus</i>    | 4  |
| North-West Prov.    | 2  | Vaal        | 3  | <i>Gyrodactylus</i>      | 2  |
|                     |    |             |    | <i>Quadriacanthus</i>    | 3  |
| Northern Cape Prov. | 1  | Vaal        | 4  | <i>Gyrodactylus</i>      | 2  |
|                     |    |             |    | <i>Quadriacanthus</i>    | 3  |
| <b>Zimbabwe</b>     |    |             |    |                          |    |
| Mashonaland West    | 2  | Zambezi     | 13 | <i>Gyrodactylus</i>      | 3  |
|                     |    |             |    | <i>Macrogyrodactylus</i> | 3  |

NL=number of localities; NF= number of fish collected;  
NS=number of species identified in the region.

## WATER QUALITY AND POTENTIAL ENVIRONMENTAL IMPACTS OF EFFLUENTS OF LAND-BASED ABALONE FARMS IN SOUTH AFRICA

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Effluent water quality was measured at nine abalone farms in two regional nodes (west and south) along the South African coastline. Total suspended solids (TSS) concentrations varied markedly as a result of tank cleaning operations. Realistic measures of TSS need to take this variability into account (median daytime TSS 10.07 mg l<sup>-1</sup> and after-hours 0.56 mg l<sup>-1</sup>). For most farms, effluent TSS exceeded the background reference level (80<sup>th</sup> percentile) and three did not comply with the 5 mg l<sup>-1</sup> standard. The dissolved components of abalone farm discharges show very little difference between working and non-working hours signals for both raw effluent and inflow-corrected effluent (e.g. inflow-corrected working 3.44  $\mu\text{mol N l}^{-1}$  and non-working 3.39  $\mu\text{mol N l}^{-1}$ ). concentrations were mostly greater than background reference levels but well below the 43  $\mu\text{mol N l}^{-1}$  standard. Inflow-corrected concentrations of nitrate, nitrite and phosphate, were low compared to and would not pose a significant eutrophication risk. Similarly, the biochemical oxygen demand measured at three of the farms was low (median 1.31 mg l<sup>-1</sup>).

Production-specific annual loads of TSS (334 kg mt<sup>-1</sup>), total N (20.3 – 38.1 kg N mt<sup>-1</sup>) and total P (3.2 – 7.5 kg P mt<sup>-1</sup>) are in agreement with what has been found for different land-based aquaculture operations. These figures translate to N-based human population equivalents, based on domestic sewage production, of 5.4 – 10.6 persons mt<sup>-1</sup> for both regions. At the broader ecosystem level, the annual TSS loads calculated from 2013 production data of 43 mt y<sup>-1</sup> (west) and 369 mt y<sup>-1</sup> (south) are, respectively, 0.35% and 2.8% of that estimated to be produced by kelp beds through natural erosion of fronds. Similarly, the dissolved inorganic N loads of 1.9 mt N y<sup>-1</sup> (west) and 9.4 mt y<sup>-1</sup> (south) are trivial by comparison with nitrate advected into the coastal zone during upwelling. Local abalone farms have a relatively high specific C footprint - conservatively about 44 kg CO<sub>2</sub> kg<sup>-1</sup> production. The present findings support a relatively low potential impact of farm effluents in this coastal upwelling environment.

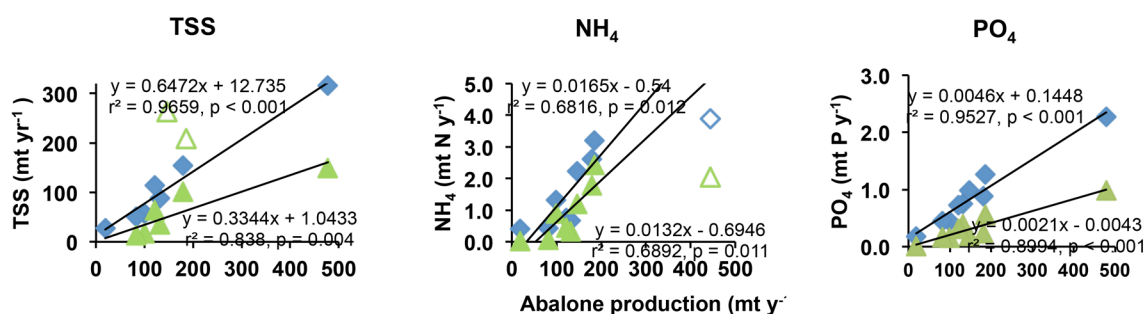


Fig 1. Mean annual discharge rates of TSS and the dissolved components; NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup> for each farm, as related to abalone production. Data are presented uncorrected (blue diamonds) and corrected (green triangles) for inflow concentrations.

## NUTRIENT DIGESTIBILITY OF EDIBLE INSECTS FOR PACIFIC WHITE SHRIMP (*Penaeus vannamei*)

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Fishmeal has been a major dietary protein ingredient for Pacific white shrimp, *Penaeus vannamei* due to its more balanced source of indispensable amino acids, higher vitamins, minerals and essential n-3 fatty acids than most of the other common ingredients used. In Thailand, the fishmeal inclusion level in commercial diet is moderately high (15–25%), which makes Pacific white shrimp as one of the top consumers of fishmeal. A variety of feed ingredients have been assessed digestibility in the diet of shrimp, such as vegetable and animal proteins. Vegetable proteins, such as soybean meal are limiting in sulphur amino acids (methionine and cysteine) and still contain some antinutrients (trypsin inhibitor, haemagglutinin, and antivitamin). Regarding sources of animal origin, most of them are forbidden by prescription of food security. Edible insects are a natural renewable resource used as food by humans. Since ancient times, insects have been one alternative protein source used to compensate for the periodic scarcity of major protein sources.

The aims of this study are: (1) to determine the nutritive values of the most common edible insect species of Thailand (silkworm, *Bombyx mori*; cricket, *Gryllus bimaculatus* and giant mealworm, *Zophoba morio*) and (2) to evaluate the digestibility of insects in the diet of Pacific white shrimp. A 4-week digestibility trial was conducted in a static water system with the initial weight of 5.0–6.6 g each. Each experimental diet was fed to three replicate groups of shrimp to the visual satiation twice daily (08:30 a.m. and 16:30 p.m.). After feeding, uneaten feed and residue were siphoned out and water was exchanged at 30–40%. Feces were collected from each tank within each dietary treatment prior to the next meal in 28 consecutive days. Based on the results, the edible insects had moderately high protein content (45–55%) and lipid (28–47%), moreover cricket and giant mealworm had higher in nitrogen free extract (NFE) than silkworm. The apparent digestibility of protein and lipid of edible insects were between of 65–72 and 83–91%, respectively.

**TABLE 1: Formulation of experimental diets fed to Pacific white shrimp (%)**

| Ingredient                     | Reference | Silkworm<br>( <i>Bombyx mori</i> )* | Cricket<br>( <i>Gryllus bimaculatus</i> )* | Giant mealworm<br>( <i>Zophoba morio</i> )* |
|--------------------------------|-----------|-------------------------------------|--|---|
| Local fishmeal, protein 55     | 60.0      | 42.0                                | 42.0                                       | 42.0  |
| Test ingredient*               | 0.0       | 30.0                                | 30.0                                       | 30.0  |
| Fish solubles                  | 1.0       | 0.7                                 | 0.7  | 0.7   |
| Wheat flour                    | 30.0      | 21.0                                | 21.0                                       | 21.0  |
| Fish oil                       | 3.0       | 2.1                                 | 2.1  | 2.1   |
| Monocalcium phosphate          | 2.0       | 1.4                                 | 1.4  | 1.4   |
| CaCO <sub>3</sub>              | 2.0       | 1.4                                 | 1.4  | 1.4   |
| Vitamin-mineral premix         | 2.0       | 1.4                                 | 1.4  | 1.4   |
| Cr <sub>2</sub> O <sub>3</sub> | 0.5       | 0.5                                 | 0.5  | 0.5   |

\* Each test ingredient was included by 70:30 (reference: test ingredient)

**TABLE 2: Proximate compositions and apparent digestibility coefficients of selected edible insects (%)**

|  | Silkworm<br>( <i>Bombyx mori</i> ) | Cricket<br>( <i>Gryllus bimaculatus</i> ) | Giant mealworm<br>( <i>Zophoba morio</i> ) |
|--|------------------------------------|---|--|
| <u>Proximate composition</u>               |                                    |   |  |
| Dry matter                                 | 21.4                               | 31.9                                      | 33.7                                       |
| Protein                                    | 49.5                               | 55.1                                      | 45.8                                       |
| Lipid                                      | 47.4                               | 28.5                                      | 40.7                                       |
| Ash  | 1.0                                | 1.4                                       | 1.2  |
| Nitrogen free extract <sup>a</sup>         | 2.1                                | 15.0                                      | 12.3                                       |
| <u>Apparent digestibility coefficients</u> |                                    |   |  |
| Protein                                    | 70.6                               | 72.9                                      | 65.6                                       |
| Lipid                                      | 89.3                               | 91.7                                      | 83.0                                       |

<sup>a</sup> Nitrogen free extract (NFE) = 100 – (protein + lipid + ash)

**GROWTH OF JUVENILE DIPLOID AND TRIPLOID SALMON (*Salmo salar*) FED A STANDARD DIET AND AN EXPERIMENTAL DIET CONTAINING HIGH PROPORTIONS OF HYDROLISED PROTEINS**

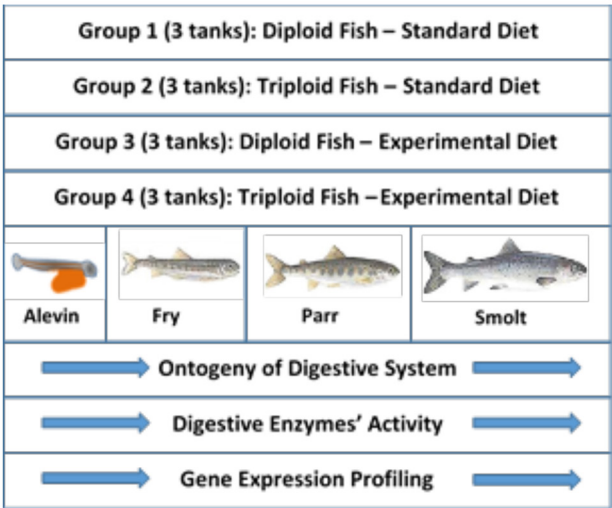
Velmurugu Puvanendran\*, Stefano Peruzzi<sup>1</sup>, Inger-Britt Falk-Petersen<sup>1</sup>, Lars Olav Ulleberg<sup>1</sup>, Fride Tonning<sup>1</sup>, Ørjan Hagen<sup>2</sup>, Jorge Fernandes<sup>2</sup>, Silvia Martinez Llorens<sup>3</sup>, Guido Riesen<sup>4</sup>, Rudi Ripman Seim<sup>5</sup>

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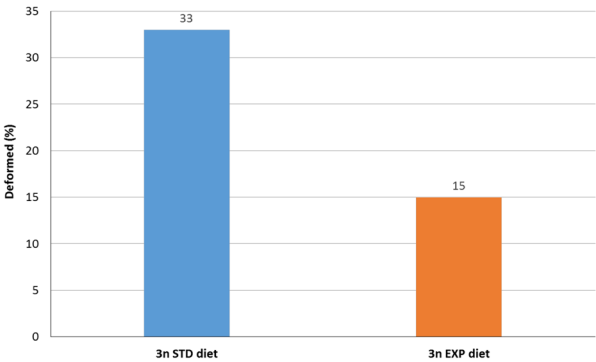
Recent studies have revealed significant differences in gut morphology between diploid and triploid fish raised on the same diets and under similar conditions (Peruzzi et al., 2014). Morphological differences in the digestive system induced by the treatment or ploidy status could have an influence on nutrient utilization and performance capacity in triploids compared to diploids. In this work, we compared the performance of diploid and triploid salmon (produced by pressure shock) fed with an experimental diet containing high proportions of fish protein hydrolysate over a 38 weeks feeding trial. Results were analysed in terms of fish survival and growth as well as muscle enzyme activities (protein turnover), and skeletal malformations.

Overall, survival was higher ( $p<0.05$ ) in fish fed standard diet than in those fed experimental diet, being 92% and 88% respectively, and with no differences between ploidy groups. At the end of the experiment, fish growth (body weight and length) was highest in triploids fed a standard diet with no significant differences among the remaining experimental groups. Condition factor varied among groups throughout the experiment and was generally lower in triploid than in diploid fish with no effects of diet. Data on muscle enzyme activities showed variations among groups at some sampling points. Percentages of vertebral deformities were very low among diploids and highest in triploids fed a standard commercial diet. Within triploids, the incidence of deformities in fish fed the experimental diet was nearly half of that reported for those fed a standard diet.

Under our experimental conditions, partial dietary replacement of fishmeal by a protein hydrosilate (CSPC) did not improve growth in triploid Atlantic salmon between start feeding and the end of the smoltification period. Initial higher mortalities in diploid and triploid fish fed the experimental diet could be ascribed to water stability of small pellets (0.5-1.0mm) related to lower binding properties as shown during their manufacture. Fish growth (bulk weight) was lower in fish fed the experimental diet in the initial phase of the feed trial but the diet containing high proportion of protein hydrolysed protein sustained growth during the end of the experiment. Interestingly, there was a positive effect of the experimental diet on the incidence of skeletal deformities among triploid fish.



Percentage deformed fish/group



## SHORT AND LONG-TERM EFFECTS OF EARLY AND LATE WEANING ON ATLANTIC COD, *Gadus morhua*

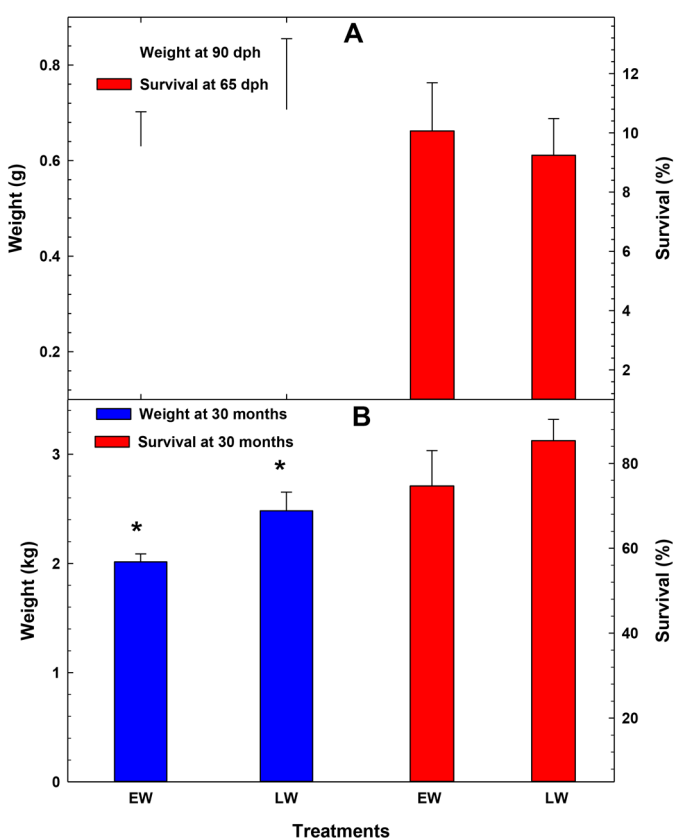
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In marine finfish larviculture, live feed is essential during the first feeding stage to ensure better growth and survival. However, live feed production is labour intensive thus; fish farmers tend to wean the larvae into dry feed at earliest possible age. We evaluated the short- (1-180 days post hatch) and long-term (at 30 months post hatch) effects of early and, late weaning on Atlantic cod from hatch to slaughter.

Gametes were collected by hand stripping and were fertilized using standard dry fertilization method. Eggs were incubated in 25L upwelling incubators at 4°C in a flow-through system. Newly hatched larvae (15,000) were transferred to six 190L circular fiberglass tanks and the water temperature was kept at 10°C. Three of the tanks kept at an early weaning (EW) regime where larvae were fed with enriched rotifers from 2-35 days post-hatch (dph) and weaning onto dry feed started at 21 dph and completed at 35 dph. Three other tanks were kept at a late weaning (LW) regime where larvae were fed with enriched rotifers from 2-29 dph followed by enriched *Artemia* from 25-56 dph and weaning onto dry feed started carried out from 45-56 dph. At the completion of weaning, both groups were fed with same dry diet until the end of the experiment. At 90 dph, the larval survival was measured by counting the individuals from each tank. The number of fish kept in each tank from day 90 to 190 dph was standardized to 300 to minimize density related effects on growth. At 190 dph, weight of 50 fish from each tank was recorded and were tagged with PIT tags and reared in the same tank until transferred to sea cages. At the end of 30 months post hatch, weight of the fish was recorded.

Our results showed no significant short-term effects of weaning method on growth and survival of Atlantic cod (Fig. 1A). No significant differences also found in occurrence of skeletal deformities of fish from EW (10%) and LW (8.6%). However, fish from LW showed a significantly higher body weight ( $p < 0.012$ ) compared to fish from EW at 30 months (Fig. 1B). Our results showed that early weaning will affect the growth of cod and we recommend using a late weaning method for cod larvae.



**Fig. 1: Effects of early weaning (EW) and late weaning (LW) on growth and survival of Atlantic cod A) at 65/90 dph and B) at 30 months.**

**FAMILY DIFFERENCES ON SEXUAL MATURATION AND ITS CONTRIBUTION TO PERFORMANCE OF ATLANTIC COD, *Gadus morhua***

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Early maturation has been seen as one of the biological bottlenecks of commercializing Atlantic cod culture. To overcome the bottleneck, production of sterile fish through triploidy and other molecular techniques have been attempted. Although studies have been carried out on triploid performance of Atlantic cod, no studies have been conducted to see family basis for triploid performance. We produced 29 triploid (3N) sibling families using standard hydrostatic pressure technique of newly fertilized eggs with parallel untreated diploid (2N) families. Larvae were reared in separate tanks using standard rearing protocols until reaching 20g and were PIT tagged. PIT tagged juveniles were transferred to sea cages in duplicate. At 34 months post-hatch, all the fish were sampled and body weight, liver weight and gonadal weight were recorded.

Triploidy analysis showed that only 8 families were 100% 3N and another 7 families had above 90% triploidization. Thus, only these 15 families were used in the analysis.

Results showed that fish from 3N families had significantly smaller gonads than fish from 2N families, but 2N families were heavier than the 3N families (Fig. 1). Ranking of body weight and gonadal weight of the families from 2N and 3N showed differences in performances among families (Fig. 1).

Significant family differences existed between 2N and 3N families in gonadal development, especially for the females, at 34 months (Fig. 2).

Our result highlight the need for considering a parallel strategy for 3N family selection within the conventional 2N breeding program to exploit the existing variation in 3N performance.

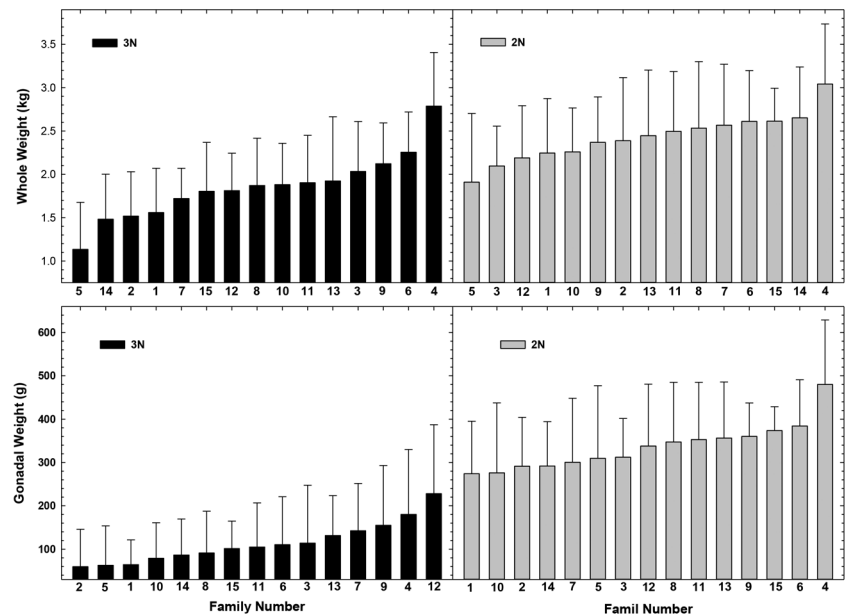


Figure 1. Family-wise ranking of whole body and gonadal weight of 2N and 3N Atlantic cod.

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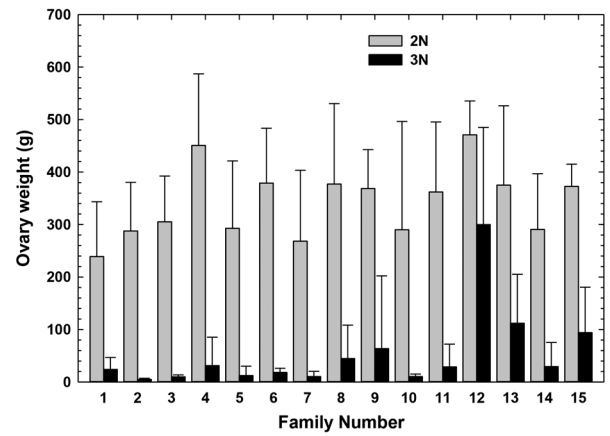


Figure 2. Comparison of ovary weight of 2N and 3N Atlantic cod



## HEMATOLOGICAL CHANGES IN *Piaractus mesopotamicus* ALLOXAN-DIABETICS CHALLENGED WITH *Aeromonas hydrophila*

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Pacu (*Piaractus mesopotamicus*) as a model for the study of alloxan-induced diabetes has advantages over other animal models, especially due to length, carbohydrate-rich diet and its rusticity. The present study aimed to elucidate the hematological alterations in *Piaractus mesopotamicus* alloxan-diabetics challenged with *Aeromonas hydrophila*.

For this, 350 pacus ( $\pm 110$ g) were distributed into 2 groups, alloxan-diabetic and non-diabetic. Each group was distributed into two new groups, control (injected with saline) and inoculated with *A. hydrophila*. The last group was evaluated at 1, 3, 6 and 9 hours after challenge. After euthanasia with benzocaine, samples were collected for hematological variables.

It was observed that hematocrit, hemoglobin, number of erythrocytes, CHCM and HCM decreased in alloxan-diabetic fish probably related to the decrease of the number and length of the erythrocytes attributed to iron consumption by bacteria and sequestration by alloxan treatment. However, these fish also had higher levels of leukocytes, granulocytes and monocytes (Figure 1), probably related to the exacerbated increase of the leukocyte adhesion in the vascular endothelium, impairing the immune response.

The results indicate that the process of induced diabetes and blood count present adequate characteristics to make this fish a model of study of this disease.

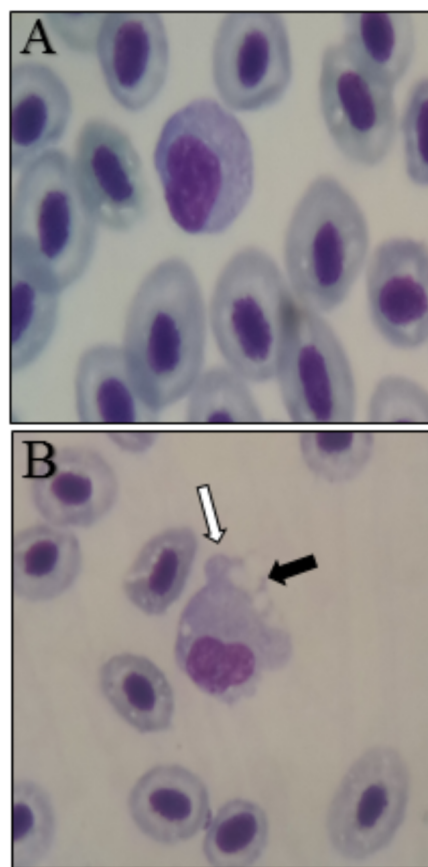


Figure 1. Young (A) and activated (B) monocytes with cytoplasmic vacuolization (black arrow) and projections of cytoplasm (white arrow).

## USEFULNESS OF PHYTOBIOTIC FOR SAPROLEGNIASIS CONTROL IN FRESHWATER FISH

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Saprolegniasis caused by species of the genus *Saprolegnia* is one of the main diseases in farmed freshwater fish, causing important economic losses in aquaculture industry worldwide. Several compounds have been evaluated for the control of this disease with different results. The goal of this study was to investigate a phytobiotic compound designed by Lipidos Toledo S.A. as an effective option against the saprolegniasis on rainbow trout (*Oncorhynchus mykiss*). The study involved two parts: 1) testing the fungistatic effect of the candidate substance and 2) evaluation of the candidate substance before and after exposure of the fish to the fungi. The administration of phytobiotic (2 baths per week during 15 days) was compared with one negative control, which received sterile saline solution, and with one positive control which received Bronopol, a product with known antifungal activity. The capacity of the phytobiotic to inhibit fungi growth was evaluated by broth microdilution method using antimicrobial concentrations ranging from 5000 to 9.8 ppm. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobial agent able to completely inhibit the growth of the fungi. *Saprolegnia* spp. (three strains isolated in Chile and one strain isolated in Spain) were sensitive to the phytobiotic compound with MIC ranging between 156 to 625 ppm. The MIC of Bronopol for *Saprolegnia* isolates ranged from 78.1 to 312.5 ppm. Bath administration of phytobiotic to fingerling rainbow trout before infection with *Saprolegnia* allowed to reduce fungi associated mortality with respect to that observed in control fish. Survival rates of 63.3% and 53.3% were observed in fish infected with *Saprolegnia* after application of the phytobiotic (30 ppm) and Bronopol (40 ppm) by bath. In conclusion, the use of phytobiotic could be an alternative and friendly solution to chemical substances for the control of saprolegniasis in freshwater fish.

## **CHALLENGES FACING AQUACULTURE POSTGRADUATE CURRICULUM DEVELOPMENT AND IMPLEMENTATION IN KENYA**

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This paper examines the existing postgraduate aquaculture curricula in Kenya, how they are developed and implemented. Capture fisheries in Kenya are rapidly declining due to high population growth rates, use of illegal fishing gears, over exploitation of the fisheries resources and a number of environmental factors which threatens the nutritional food security in the country.

Aquaculture promises to be the most suitable option yet efforts to increase aquaculture food production in the country have remained low. Steady increase in aquacultural food production depends heavily on well qualified resource use managers. Fit for purpose curriculum, the way curriculum is implemented including cascading the knowledge and skills to both students and fish farmers. High dependence on individual University Senate in curriculum development, Lack of aquaculture infrastructure, poorly equipped laboratories, and inadequate stakeholder participation are among the challenges. The study recommends a centralized national curriculum development supervised by the national regulator of university education which would give rise to a common approach in providing knowledge and practical skills to postgraduates and the undergraduates and subsequently to the fish farmers.

## **AQUACULTURE DEVELOPMENT AT KAFR EL SHEIKH CASE STUDY**

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Kafr El sheikh is located in the middle east of the Nile Delta, where it overlooks the Mediterranean Sea Shore in the north and the River Nile (Rashid Branch) in the west. It is mainly agricultural governorate and the agricultural cultivated area forms about 62% of its total area. Moreover, Kafr El Sheikh is leading in regard to fish activities.

The governorate has a share of Mediterranean Sea, Lake Burullus, and aquaculture establishments represented in fish hatcheries, fish nursery station, three governmental fish farms and very many private fish farms.

Thus, fishery activities whether in capture fishery or in aquaculture called for various levels and types of work opportunities including fishermen, fish traders, boat builders, fish farmers, fish feed producers and others.

Lake Burullus is characterized by its high productivity as its production amounted 63,980 tons in 2014 of the main species are (tilapia, shrimp, crap, mullet and catfish). In addition to fishery, Lake Burullus enjoys an international importance for birds under Ramsar convention.

The remaining sources of capture fishery include Mediterranean Sea fishery as well as River Nile fishery.

In regard to aquaculture, Kafr El Sheikh is by far the leading aquaculture governorate with its private fish farms, governmental farms, cages and integrated rice-fish culture. In fact, the first all-male tilapia hatchery was established in Kafr El Sheikh back during 1990s. Equally important, the first long-term 6-month training program took place in 1980 with the support of FAO.

There are several feed mills producing fish feed as required by the growing aquaculture operations in the governorate and beyond. Also, there is a wholesale fish market that serves fish producers as well as retailers in Kafr El Sheikh as well as the delta region. About 41.9% of total fish in Egypt is produced in Kafr El Sheikh; out of which 539,564 tons from aquaculture which represents 47.5% of total Egyptian aquaculture in 2014.

In regard to academic and aquaculture research, there is an aquaculture college that belongs to kafr El Sheikh University.

## FEEDING AND NUTRITION OF JUVENILE COBIA *Rachycentron canadum*: EVALUATION OF PRACTICAL FEEDS IN FLOATING NET CAGES, COMPARISON OF DIFFERENT DIETARY FISHMEAL REPLACERS WITHIN INDOOR TANKS, AND ESTIMATION OF ESSENTIAL AMINO ACID REQUIREMENTS

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Two feeding trials were conducted with juvenile cobia *Rachycentron canadum* at the Oceanographic Institute, University of São Paulo, Brazil: (1) a 10-week feeding trial conducted within floating net cages to test the nutritional efficacy of different dietary feeding regimes ranging from the use of trash-fish, in-house formulated feeds, to dry commercial extruded marine fish feed; and (2) a 10-week feeding trial conducted within indoor water-recirculated tanks to test the nutritional efficiency of different potential dietary fishmeal replacers within dry in-house prepared diets.

Different commercially-based diets were tested within floating net cages, including a trash-fish control diet, a semi-moist diet composed of trash-fish and a binder premix, an in-house dry formulated diet, and one commercially available extruded marine fish feed. In the case of the indoor tank-based feeding trial, different practical-type diets were formulated and manufactured containing 50% crude protein from different animal and plant protein sources, including a national fishmeal diet, a salmon fishmeal diet, a poultry-by-product meal diet, a soy protein concentrate meal diet, and a combination of poultry-by-product meal and feather meal diet. In addition to the above different protein sources, a diet was also tested without supplemental taurine together with a dry commercial fish feed. Juveniles cobia were randomly stocked in both systems and hand-fed *ad libitum* two times a day. Feces were recovered by the fecal stripping technique in anesthetized individuals, in order to assess dry matter and crude protein apparent digestibility coefficient.

Generally, fish performance was superior in the net-cage feeding trial compared with the indoor water-recirculated tank trial. Fish weight gain in both feeding trials were not significantly different between the experimental diets, with the exception of fish fed the trash-fish control diet which displayed the best growth response and feed performance. Overall, the fish growth and performance of the experimental diets were very similar, showing that the alternative ingredients could be included and replace part (50%) of the fishmeal component. Apparent crude protein digestibility coefficients of over 80% were obtained for all diets from both trials. The results from both trials concluded that the cobia requires practical diets with high levels of crude protein and lipid, and the inclusion of alternative plant-based and terrestrial animal protein sources was possible. Comparing the estimated essential amino acid (EAA) requirements of juvenile cobia using two different approaches (protein accretion and E/A ratio), an underestimation of the EAA requirement values was noticed when lysine was used as the reference EAA. The quantitative EAA requirement values estimated by the protein accretion method was highly correlated to the average of each of the EAA requirement for the species of carnivorous fish reported in the literature, and could be recommended for formulation of commercial feed for cobia. Using amino acids profile to set basis for dietary amino acids profile and requirements is a viable technique and may bring additional benefits of formulating diets eliciting higher feeding efficiency and reduced nutrient loss and waste and metabolites excretion.

## PATHOGEN MONITORING IN INDOOR SYSTEMS OF TILAPIA AQUACULTURE IN IRAN

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In recent years, aquatic diseases have damaged aquaculture industry in different areas of the world. Although tilapias are known as resistant fish against different pathogens, but there are some reports about infectious diseases of tilapia. Tilapia aquaculture in Iran is in the beginning stage. So, planning for hygiene observations and preventing of disease prevalence must be considered. The aims of the present study were hygiene monitoring of tilapia hatchery and indoor systems, diagnosing infectious diseases of tilapia and alignment of instruction for hygiene management of tilapia aquaculture.

Tilapias are the second farmed food fish in the world. Tilapia production has rapidly expanded in the world in recent years.

Researches about tilapia were started in Iran from November 2008 and different aspects of breeding, culture and nutrition were considered.

In the present study, tilapia hatchery and indoor systems of National Research Center of Saline water Aquatics were monitored for infectious disease from October 2012 to February 2014. Unusual locomotion/feeding or uncommon signs in the shape or body surface of cultured tilapias, fries and eggs or exceed mortalities were mentioned for bacterial, fungal, parasitic or viral pathogens.

Infectious agents including bacteria, fungi, parasites and viruses were considered.

Recorded pathogens were rarely observed during the study. No viral agent was diagnosed.

Much attention must be paid for hygiene monitoring and instruction performance to prevent infectious diseases.

## POTENTIAL OF LOCAL ICTHYOPHAGIC FISH SPECIES IN THE OPTIMIZATION OF MULTITROPHIC FISH FARMING SYSTEM IN MADAGASCAR

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From 1914 to 1975 many exotic fish species have been introduced in Madagascar, with the purpose of promoting the intensification of continental fish farming. Nowadays, the two main continental fish species reared in Madagascar are the common carp (*Cyprinus carpio*) and the Nile tilapia (*Oreochromis niloticus*), in monoculture or polyculture. This strategy was motivated by the broad amount of information on the biological bases for the aquaculture of these exotic species, in contrast to the limited knowledge on Malagasy species. However, following these serial introductions and the destruction or modification of habitats caused by anthropic activities and environment degradation, Malagasy indigenous ichthyofauna declined. Such decline has raised serious concerns from scientists and decision-makers, who are increasingly interested in the possibility of using local species for fish farming.

Agro-ecological intensification of fish farming constitutes a real stake for development and food security in rural areas of Madagascar. For tilapia in particular, production systems suffer from uncontrolled fry production, which leads to overpopulation and slow growth due to limited trophic resources. Controlling the proliferation of tilapia fry by an indigenous piscivorous fish can be advantageous, also for promoting biodiversity conservation, however knowledge about Malagasy fish is scarce.

A first work on species inventory and characteristics has been proposed by Kiener (1963), and then complemented (among others) by Reinthal & Stiassny (1991) and De Rham (1996). These works permitted to establish a list of known species present in Madagascar. The aim of the present study was therefore to identify among this list some local, and ideally endemic, piscivorous species to be associated with tilapia in polyculture, in order to control its reproduction. To achieve this aim, the following components were evaluated (Fig. 1):

- Multicriterial evaluation of fish species to be reared in polyculture with tilapia in Madagascar
- Study of the biology and ethology of the selected species in microcosm and mesocosm for their domestication
- Evaluation of the predation capacity of selected species

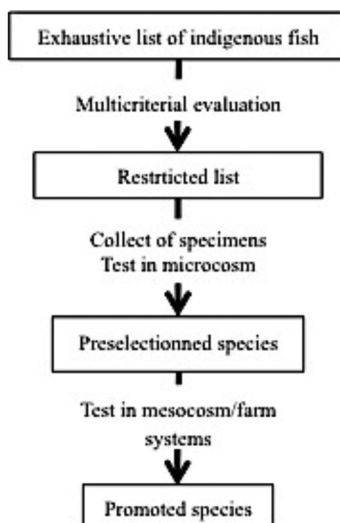


Fig. 1: Stepwork for indigenous fish selection



## LOSES AND WASTES OF THE FRESHWATER FISH SECTOR IN ANTANANARIVO, MADAGASCAR

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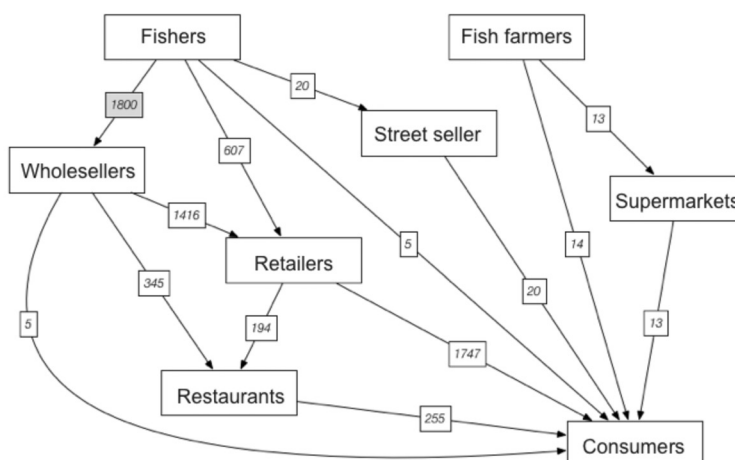
The GloFoodS project “P-G&City”, meaning losses and wastes in cities, aims to develop a common method to study the territorial metabolism and its adaptation to the urban context. This method use an analysis of losses and wastes reduction initiatives or valorization, through modifications of biomass flows. The main project is focused on five cities (i.e. Dakar, Montpellier, Chicago, Antananarivo and Hanoi), while fish is only analyzed in Antananarivo. Indeed, with about two million inhabitant, this city is a central place of freshwater fish consumption in Madagascar, while the greatest lakes from the surrounding regions guarantee fish supply.

Losses and wastes were qualified and quantified along the fish sector and at the level of the different actors. This study was mainly based on material flow analysis method that consists in evaluating material (1) entering into the system, (2) stored in the system, (3) leaving the system and (4) losses and wastes.

Work steps description:

- Identification of actors and steps of the freshwater fish distribution sector
- Quantification for each actor of volumes purchased, sold, stored as well as losses and wastes
- Extrapolation of these volumes to the urban system of Antananarivo and mapping of flow and material

Categorization of products quality levels was realized. Also, following surveys carried out on collectors, fishers and markets, mapping of the freshwater fish sector and quantification of fluxes was completed (Fig. 1). The main difficulty encountered was linked to the extent of the informal and to the inaccuracy of resulting data. Indeed, estimations based on household surveys suggest 10-fold higher fish consumption than official statistics. It also turns out that losses on consumable fish parts are very limited although carcass, scales and viscera account for about half of the wet weight.



1  
: Mapping of freshwater fish sector material flow

## COMPARATIVE EVALUATION OF METABOLIC ENZYMES ACTIVITIES IN DIFFERENT TISSUES OF *Pangasianodon hypophthalmus* (SAUVAGE, 1878) FINGERLING REARED AT HIGHER TEMPERATURE

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A 60-day feeding trial was conducted to study the effect of higher temperature (32°C) in comparison to ambient temperature on different metabolic enzyme Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Adenosine triphosphatase (ATPase), Acid Phosphatase (ACP) & Alkaline Phosphatase (ALP) activities in different tissues of *Pangasianodon hypophthalmus* (Sauvage, 1878). AST activity differ significantly ( $P < 0.05$ ) in case of liver, however, temperature do not significantly affect the AST activity in case of muscle ( $p > 0.05$ ). The ALT activity differ significantly ( $P < 0.05$ ) in case of liver and muscle at different temperature. ATPase activity (at 37°C) in gill do not differ significantly ( $P > 0.05$ ) whereas ATPase activity in liver at different temperature differ significantly ( $P < 0.05$ ). ACP activity does not vary significantly at different temperature whereas ALP activity varies significantly at different temperature in liver and intestine tissues. The present study clearly shows that there is temperature dependent change in activity of different metabolic enzyme in different tissue studied in comparison to the control at ambient temperature. The finding suggests that the metabolic responses are variable and accordingly, temperature impacts on the growth performances of this new candidate species for aquaculture.

## USING THE IMMUNOSTIMULANTS AS A ENVIRONMENTAL FRIENDLY IN CONTROLLING THE VIRUS DISEASES ON THE TIGER SHRIMP (*Penaeus monodon*) AQUACULTURE IN INDONESIA

Rantetondo

Shrimp is the main exporting product from fishery sector of Indonesia. In 1991, Indonesia was the second rank producer of shrimp next to Chinese by total production more than 660.000 metric tons. Since 1993, however, the production of shrimp has been decreasing, dramatically. (decrease about 84.84 %) The main cause of this decreasing are viral and bacterial. Currently has been used many kinds of antibiotic such as chloramfenicole, ampicillin, gentamicin, neocine tetracycline, penicilline G. and others. , to control disease, especially bacterial disease Nevertheless, in the reality many of antibiotic are referred [as] generate strain new bacterium that resistant's is not effective in battling the disease. Nowadays, prevention and disease controlling become main priority in maintaining continuity of shrimp culture industry. Immunology of shrimp is a key element in determining strategy for disease control at shrimp culture. The usage of immunostimulants for example  $\beta$ -glucan and lipopolysaccharide (LPS) can improve nonspecific immune response because it can improve phagocytosis activity from cellular defence, that have : effect on bactericidal activity In the other hand the shrimp will resistant to viruses diseases Using the immunostimulants in the shrimp culture for controlling the disease is a friendly environment because materials of the immunostimulants easy to reduce in the environments.

### INTRODUCTION

Shrimp is the main exporting product from fishery sector of Indonesia. In 1991, Indonesia is the second rank producer of shrimp next to Chinese by total production more than 660.000 metric ton (Anonymus, 1991 in Prayitno, 1997). This succeeded is a short period because there is a failure of shrimp culture since 1989. The biggest failure of shrimp culture was occurred in 1993, where the production of shrimp downwardness till reaches 100.000 metric ton (Anonymus, 2002). According to Dahuri (2001), production of shrimp Indonesia in 1998 only around 167.550 metric ton, so the percentage of increase/year from 1994-1998 only 5.56%. In 2000 Indonesian shrimp productions was only 97.710 metric ton. (Anonymus, 2002). In comparison with production in 1991, there was a degradation production account for 85.19 %. The main cause of shrimp failure culture was disease virus outbreaks and conducting culture management that unfavorable and ecological potency degradation, as a consequence decreasing of shrimp resistance to diseases. Loss that generated by shrimp disease reaches 300 billion rupiahs per year for all ponds area in Indonesia (Rukyani and Sunarto, 1998). However, grace on the encourage of fish farmers and the applied of Shrimp Culture Health Management the production of shrimp by brackish-water ponds have been increased from 242,500 ton in 2004 to 300,000 ton in 2005 (Putro, 2005).

In 2008 the production of aquaculture include shrimp increase up to 1690,000 tons, just the fourth rank after China with the total production of 32736,000 tons ( Dahuri, 2011).

In 2006 the target of shrimp production of Indonesia was 350,000 ton. To get this target the government of Indonesia introduced the new species *Vannamei* (*Litopenaeus vannamei*) from Hawaii (US). This new species was cultured in both traditional and semi intensive brackish-water ponds to increase the shrimp production that decrease due to the diseases outbreaks. Actually *Litopenaeus vannamei* is not resistance against the virus the same as tiger shrimp *P. monodon* (endogenous species). Recently, Myo Necrosis Virus has been reported to cause mortalities *L. vannamei* reared in brackish-water ponds in Situbondo (East Java) and others place in Indonesia. Therefore, the research on the genetical engineering to produce the broodstock that resistance to disease is doing in some institutes, especially to improve the genetic of the endogenous species tiger shrimp *P. monodon*.

To control disease, especially bacterial disease, currently has been used some kinds of antibiotic such as chloramfenicole, amphisilene, gentamisin, neosine, tetracycline, penicilline G. Nevertheless, in the reality many of antibiotic are referred [as] generate strain new bacterium that resistant's is not effective in battling the disease. According to Rosily (1987), there are about 200 new strains from bacterium *Vibrio* that resistant to more than two antibiotics. In consequence, it's required to look for new methods in effort for disease problem. One of way to control disease is by increasing immune system of shrimp.

(Continued on next page)

The data of shrimp export from 2000 to 2004 as shown in Table 1 while up to date data export of shrimps from Indonesia and locally (South Sulawesi) as shown in Table 2. Today situation of shrimp export from Indonesia as mention in the Sindo newspaper (March,19, 2015) as follows

Indonesia was ranked below India worth USD91.4 million or 22.19%, followed by Ecuador worth USD51.1 million or 12.41%. While Thailand and Vietnam respectively only worth USD44.3 million or 10.7% and USD34.2 million or 8.3%. The most preferred shrimp products US citizens were peeled frozen warm water shrimp. Trends in US demand to fishery products from Indonesia are currently wide open ( Newspapers SINDO March 19, 2015).

Nowdays, prevention and disease control become main priority in maintaining continuity of shrimp culture industry. Immunology of shrimp is a key element in determining strategy for disease control at shrimp culture. In consequence, direct research at development come [from] (experiment) to evaluate and monitor impenetrability level shrimp must be conducted (Bacheré, 2000).

According to Secombes (1994), the usage of immunostimulant for example  $\beta$ -glucant and lipopolysaccaride (LPS) can improve nonspecific immune response because it can improve phagocytosis activity from cellular defence, that have : effect on bactericidal activity.

This immunostimulant suggest as a friendly environment due to the materials used will preven the disease and do not destroy the environments. The materials contens of the immunostimulants easy to reduce so that the immunostimulants do not contaminate the shrimp. Immunostimulants  $\beta$ -glucant can increase the survival rate and immune response in tiger shrimp *P. monodon* cultured in fiber glass tanks (Rantetondok,2000).

The research on the effect of two immunostumulants that is  $\beta$ -glucant and lipopolysaccaride (LPS) on respiratory burst conducted by the authors indicated that there was the strong relationship between respiratory burst and survival rate of the tiger shrimp. Dose LPS 30 g/kg feed showed the highest both in activity of respiratory burst and survival rate of the tiger shrimp when fed in net enclosed during the juvenile growing (called "*Pembantuan*")n brackish-water ponds. Immune response can be seen in the increase of the respiratory activity of cell of the shrimp (Rantetondok, *et al*, 2004). The method of shrimp (invertebrate) to develop the immune-system called ProPO System as shown in Fig. 1 (Modified by Alday-Zanz, 1965).

## MATERIALS AND METHODS

This research conducted by review the literature (Literature Study) that connected by the title include the result of the author (s) research and the newspaper cited.

Data collected from Indonesian Fisheries Statistic (National) and from Marine and Fisheries of South Sulawesi and others from author`s Ph.D dissertation.

Research on using  $\beta$ -glucant and lipopolysaccaride as a immunostimulant for disease prevention in tiger shrimp (*Penaeus monodon* Fabricius) have been conducted both *in door tank* and *out door* (brackish-water ponds).

### The Methods of Immunostimulants Applied to Shrimp

**$\beta$ -glucant** : Immunostimulant was given to shrimp through mix with crumble feed with 3 doses each : 5 gr/kg feed, 10 gr/kg feed and 15 gr/kg feed . Feed amount that given is 10% from shrimp biomass weight per day and decreasing according to age of shrimp, at the end of research only 5% from shrimp biomass weight. Feed with immunostimulant was given only at first day once a week, and the observation of the effect immunostimulant was conducted at week 2, 4, 6, 8th. Shrimp sampling was conducted every week to weight shrimp biomass that will be a reference to count amount of given feed.

**Lipopolysaccharide (LPS)** : was given to shrimp through mix LPS with crumble feed with 3 doses each 10 gr/kg feed, 20 gr/kg feed and 30 gr/kg feed. Immunostimulant giving method through shrimp feed is precisely the same with at B-glucant. Shrimp that has been stimulated its immune system by using both types of immunostimulant was challenged infected by pathogen bacterium *Vibrio harveyi* with dose  $5 \times 10^4$  CFUs/ml through inverted for 96 hour. (Sung *et al*,1998).

(Continued on next page)

Challenger test was conducted at the end of culture period of larvae phase at week 2, 4, 6, 8th, from each set of experiment unit to calculate survival rate. The method to calculate survival rate was by undertake shrimp sampling account for 10 shrimps of each container of research basin. Hereinafter, shrimp was put into 21 units- 1 litre glass based on replicate and type of its immunostimulant. Shrimp prior to put into glass was given disinfectant  $\text{KMnO}_4$  4 ppms for 1 hour. The water that will be used to challenge test was sterilized with ultra violet ray. Hereinafter each unit of experiment glass was infected by *V. harveyi* bacterium with dose and time such as those which have been mentioned above.

## RESULTS

### Using $\beta$ -glucant and lipopolysaccaride as a immunostimulant

The Average of Survival Rate (%) for Post Larvae of Tiger Shrimp Using Immunostimulants  $\beta$ -glucant and LPS after Challenged Test with White Spot Baculovirus(after grouping to Duncan Range Test) is shown in Tabel 3. **The Average of Survival Rate (%) of tiger Shrimp Juvenile after Challenged with *Vibrio harveyi* with concentration of  $5 \times 10^4$  CFU/ml for 96 hours is shown in Table 4.**

### Lysozyme Activity

The lysozyme activity of tiger shrimp treated by  $\beta$ -glucant and LPS is shown in Table 5.

## DISCUSSION

Research on using  $\beta$ -glucant and lipopolysaccaride as a immunostimulant for disease prevention in tiger shrimp (*Penaeus monodon* Fabricius) have been conducted inat *in door tank* and *out door* (brackish-water ponds). The results of this research indicated that both immunostimulants can enhance the immune response of shrimp to prevent the outbreaks of disease especially the White Syndrome Baculovirus or White Spot Syndrome Virus (Table 4) ( Rantetondok, 2002). This immunostimulant suggest as a friendly environment due to the materials used will preven the disease and do not destroy the enviromnts. The materials contens of the immunostimulants easy to reduce so that the immunostimulants do not contaminate the shrimp . Nowadays, prevention and disease control become main priority in maintaining continuity of shrimp culture industry. Immunology of shrimp is a key element in determining strategy for disease control at shrimp culture. In consequence, direct research at development come [from] (experiment) to evaluate and monitor impenetrability level shrimp must be conducted (Bacheré, 2000).

### Lysozyme Activity

Tiger shrimp treated by  $\beta$ -glucant and LPS indicated the increasing of the lysozyme activity which can be seen in the high survival rate of the juvinel after perform the challenger test by *Vibrio harveyi* bacteria (Table 4) (Rantetondok *et. al*, 2003).

Lysozyme is one of the indicators to decide the effectiveness of the immunostimulants or immune response of the organism. According to Ellis (1990), lysozyme has anti bacterium activity that caused lisis and can also personate opsonim. Specific substrat was found on lysozyme is  $\beta$ -(1-4) "linked" with N-acetyl-D-Glikosamin, N-acetyl-asam muramil peptidoglucant that found at some wall cell of bacterium and if it was digested will cause plasma fall to pieceses caused by osmosis pressure. When that's lysozyme at haemocyte shrimp will have the character of anti bacterium that mean that immune system of shrimp has been activated. Thus giving  $\beta$ -glucant and LPS that functioned as [the] goals from recognition of protein form (Pattern Recognition Protein) can generate power of body defence shrimp pass by improvement lysozyme. Besides direct influence as anti bacterium, lysozyme is also reported can improve phagocytosis. Lysozyme found in serum or mucus of fish especially the tissue wich enreach in leucocyte such as kidney, gaster, and limp (Engstad, *et al*, 1992).

### Immunostimulant Agents

One of the methods to increase the non-specific defence of animal in aquaculture is to apply immunostimulants such as  $\beta$ -glucant and lipopolysaccaride (LPS). This is the of one the best methods to prevent the disease in aquaculture system (Scombes, 1994). Nowadays some of antibiotics have been used to preven the bacterial disease such as Furazolidone 12 ppm, Teramicyn 450 mg/kg body weight of shrimp added to feed, and Furanace 1,3 ppm that effective as bactericidal especially to control Vibriosis (Taren, 1985). In the other hand however, the residual of these antibiotics have a side effect to shrimp and humen due to the resintence of the phatogenic bacteria such as *Vibrio* to Chloramphenicol, penicilline G. and others. The recidue of the these antibiotics can stay in the body of shrimp 2 weeks after initial treatments, and these will be harmful for humen being as a consumer whose directly use without waiting for 2 weeks afer initial of treatments. These indicated that antibiotic can react as a therapeutipic but it can be a lethal for shrimp because at the dose over the tolerant limit it can be destructed the potensial cell. Use of the antibiotic for long period can be resulted in resistance of the bacteria to some of the antibiotics. Therefore using of immunostimulants is a good choice for control disease and it is a friendly environments methods.

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$\beta$ -glucans are branched  $\beta$ -1,3 and  $\beta$ -1,6 linked polyglucoses and are major structure polysaccharide in cell walls of most fungi yeast and mycelia fungi. Higher organisms have developed various recognition mechanisms for these conservative fungal wall components.  $\beta$ -glucans are those able to stimulate non specific defence mechanism in animal as well as plants. It is well established that  $\beta$ -glucans in mammals increase the resistance against microbial infections and stimulate anti-tumor mechanism. There is now a growing body of evidence that  $\beta$ -glucans also function as a immunostimulator in fish. They thus have a potential as a feed additives to counteract in immunosuppressive states of fish and as vaccine adjuvants (Robertsen *et al.* 1994).

Fungi *Mitake (Grifora frondosa)* found in Japan is branched and  $\beta$ -1,6 linked polyglucoses and it have a potential as immunostimulator to increase non-specific defence mechanisms in human (Ganjar, 1997). Furthermore it was decided that  $\beta$ -glucans can stimulate the immune system of the body such as macrophages, natural cell killer, T cell killer (cytotoxic), T cell helper, interleukin-1, and interleukin-2 which can inhibit the growth of cancer and virus. According to Tahir (1996)  $\beta$ -glucans have an important prospect as a immunostimulator. Tiger shrimp which is treated with  $\beta$ -glucans at concentration of 0.5-1 mg/ml (immersion treatment) following the challenge test by bacteria *V. vulnificus* at concentration of  $5 \times 10^4$  CFU/ml for 12 hours indicated that there was an increasing in phenoloxidase activity of hemocyte 1 month after the initial treatments. *P. javanicus*, orally treatment by  $\beta$ -1,3 glucans (extracted from *Schizophyllum commune*) showed the increasing of phagocytic activity of hemocyte defence mechanisms against bacteria *Vibrio* sp (JICA, 1994). Trout immersed by chitosan or glucan for 30 minutes or injection for 1-3 h days before challenge test by bacteria *Aeromonas salmonicida* indicated the good defence mechanisms of this species. Injection administration (treatment) is better than the immersion (Anderson, and Siwicki, 1994).  $\beta$ -glucans are major structural polysaccharides in cell walls of most yeast, filamentous fungi and mushrooms are also secreted as a slime or mucilage by some fungi (Rosenberger, 1976 in Robertsen, 1994). From an evolutionary point of view, these structure probably represent one of the most ancient microbial cell wall components. Consequently, higher organism may have developed various recognition mechanisms for these conserved fungal wall polysaccharide early in the evolution. This may be the reason why  $\beta$ -glucans have been found to stimulate or activate the non-specific defence mechanism in a wide range of higher organisms, plants (Darvill and Albersheim, 1984 in Robertsen *et al.*, 1994). Tiger shrimp treated by Chitin in different dose can increase the chitinase activity in the ingestion canal of the shrimp. Chitinase activity increase accordingly to the increasing of chitinase percentage in feed (Fox, 1993). Extract of abalone which are a glycoprotein component can increase the phagocytic activity and chemiluminescence of kidney leukocyte when intraperitoneal injection to the rainbow trout at the dose of 20 mg/kg feed. This increasing occur 5 days after treatments and following by the increasing of defence mechanism against *V. anguillarum* attack (Secombes, 1994). Lipopolysaccharide (LPS) are O-antigen and endotoxin in Gram-negative bacteria can increase the phagocytic activity of leukocyte in sea bream *Pagrus major* and lamp macrophages in plate fish *Pleuronectes platessa* when in injection at dose of 500  $\mu$ g for 5 days and showed the increasing of higher migration compare to the control (MacArthur *et al.*, 1985). Furthermore, LPS also can stimulate directly the phagocytic in culture medium.

Bacterium cell, *Vibrio* sp killed by 0.5% formaldehyde and added in feed encapsulated can increase the survival rate in tiger shrimp larvae. It is assumed that the bacterium cell is a substance which can increase the defense mechanisms in shrimp larvae against the attack of bacteria in water (Itami, *et al.*, 1991). LPS extracted from *Cytophaga* bacteria, and injection to turbotfish, *Scophthalmus maximus* than challenge tested by the same bacteria 21 days after initial injection can decrease the mortality 0-5 % lower than control of 90-100 % mortality. Fish vaccinated showed the higher haemagglutination titer compare to the control (Al-Harbi and Austin, 1992). The result of the challenge test by the *Vibrio* spp against the tiger shrimp postlarvae (PL 20) treated for 96 hours by LPS extracted from *E. coli* showed the tendency of the more decreasing the mortality the more increasing the dose of LPS and the duration of treatment (Tahir, 1996). They thus have a potential as feed additives to increase the phagocytosis of bacteria so that it can increase defence mechanism against the microorganism in the brackish-water ponds.

### **Lysozyme**

The methods of the immunostimulants applied to shrimp is useful to improve the oral administration because this is one of the best methods beside the others methods such injection, immersion, spraying and others. Nevertheless it is needed to improve the administration of immunostimulant applied to shrimp eg. Using the microencapsulated feed for shrimp larvae and the ability of the shrimp to eat the such feed in culture medium (brackish-water ponds or net enclosures for culture the post larvae). The develop of research on the applied of immunostimulants in the field is needed in connecting to produce the seed which specific pathogen resistant. On the other hands the research on the genetic engineering to produce the seed which resistance to disease is also needed so that the production of the shrimp can be increased in Indonesia.

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## ACKNOWLEDGMENTS

The author wish to thank Rector of Hasanuddin University for her support research ant to pulish the manuscript in the International Journal. The author is also very grateful to Dr Nita Rukminsari of the same university for her crtical reading of the manuscript and as a correspondence e-mail.

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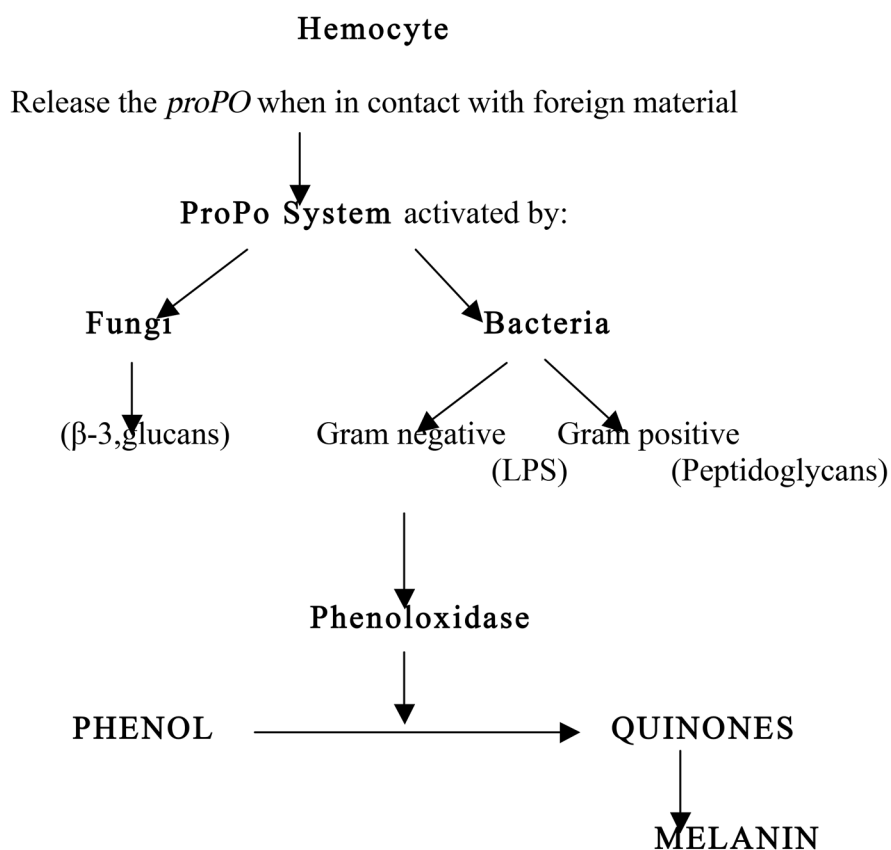
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Figure 1. Scheme ProPo System (Alday-Zanz, 1995)



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Table 1. Indonesian Shrimp Export

| Year | Volume (ton) | Value (Million US \$) |
|------|--------------|-----------------------|
| 2000 | 114,035.1    | 1003.26               |
| 2001 | 127,344.3    | 940.1                 |
| 2002 | 122,050.0    | 840.35                |
| 2003 | 134,241.6    | 852.72                |
| 2004 | 127,846.3    | 823.96                |

(Source: Indonesian Statistic, 2004)

Table 2. Indonesian Shrimp Export and South Sulawesi from 2010 -2015

|                       | YEAR      |          |           |           |           |        |
|-----------------------|-----------|----------|-----------|-----------|-----------|--------|
|                       | 2010      | 2011     | 2012      | 2013      | 2014      | 2015   |
| <b>Indonesia</b>      |           |          |           |           |           |        |
| Volume (ton)          | 145,092   | 158,062  | 162,068   | 167,565   | 196,623   |        |
| Value (US \$ 1.000)   | 1,056.399 | 1,09.674 | 1,304,149 | 1,684.086 | 2,140,862 |        |
| <b>South Sulawesi</b> |           |          |           |           |           |        |
| Volume (ton)          | 4,896     | 4,174    | 3,952     | 5,255     | 4,824     | 4,220  |
| Value (US \$ 1.000)   | 52.760    | 49,572   | 46,113    | 65,590    | 71,297    | 54,548 |

(Continued on next page)

Table 3 The Average of Survival Rate (%) for Post Larvae of Tiger Shrimp Using Immunostimulants  $\beta$ -glucan and LPS after Challenged Test with White Spot Baculovirus(after grouping to Duncan Range Test)C Note: Treatment with the same character is not a significantly difference based on Duncan test .

| Treatments          | Average | Duncan Range Test |
|---------------------|---------|-------------------|
| Glucan 5 g/kg feed  | 9.670   | A                 |
| LPS 30 g/kg feed    | 6.840   | BA                |
| LPS 20 g/kg feed    | 6.630   | BA                |
| Glucan 10 g/kg feed | 6.170   | BA                |
| Glucan 15 g/kg feed | 4.340   | BA                |
| Control             | 0.710   | B                 |
| LPS 10 g/kg feed    | 0.710   | B                 |

Table 4 the Average of Survival Rate (%) of tiger Shrimp Juvenile after Challenged with *Vibrio harveyi* for 96 hours

|                            | Treatments   |                |                 |                 |                 |                 |                 |
|----------------------------|--------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                            | Control      | Glucan         |                 |                 | LPS             |                 |                 |
|                            |              | 5 g/kg<br>Feed | 10 g/kg<br>Feed | 15 g/kg<br>Feed | 10 g/kg<br>Feed | 20 g/kg<br>Feed | 30 g/kg<br>Feed |
| <b>Average</b>             | <b>16.67</b> | <b>90.00</b>   | <b>100.00</b>   | <b>100.00</b>   | <b>90.00</b>    | <b>100.00</b>   | <b>96.67</b>    |
| <b>SD <math>\pm</math></b> | <b>12.47</b> | <b>8.16</b>    | <b>0.00</b>     | <b>0.00</b>     | <b>8.16</b>     | <b>0.00</b>     | <b>4.71</b>     |

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**Table 5.** . The average of lysozyme activity ( $\mu\text{g/ml}$ ) by the treatment (Note : LPS 30 g/kg feed cannot be determinated=UD)

| Treatments |             |                 |                 |              |                 |                   |
|------------|-------------|-----------------|-----------------|--------------|-----------------|-------------------|
| Control    | Glucan      |                 |                 | LPS          |                 |                   |
|            | 5 g/kg feed | 10 g/kg<br>feed | 15 g/kg<br>feed | 10 g/kg feed | 20 g/kg<br>feed | 30 g/kg<br>feed * |
| 1.96       | 6.59        | 7.33            | 13.69           | 5.96         | 1.99            | Ud                |
| 0.09 (SD)  | 0.05 (SD)   | .05 (SD)        | 0.57 (SD)       | 1.98 (SD)    | 0.05 (SD)       | Ud                |

\*) Note : LPS 30 g/kg feed cannot be determinated=Ud)

## USING THE IMMUNOSTIMULANTS AS A ENVIRONMENTAL FRIENDLY IN CONTROLLING THE VIRUS DISEASES ON THE TIGER SHRIMP, *Penaus monodon* AQUACULTURE IN INDONESIA

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Shrimp is the main exporting product from fishery sector of Indonesia. In 1991, Indonesia was the second rank producer of shrimp next to Chinese by total production more than 660.000 metric tons. Since 1993, however, the production of shrimp has been decreasing, dramatically.(decrease about 84.84 %) The main cause of this deceasing are viral and bacterial.

Currently has been used many kinds of antibiotic such as chloramfenicole, ampicillin, gentamicin, neocine tetraciline, penicilline G. and others. , to control disease, especially bacterial disease Nevertheless, in the reality many of antibiotic are referred [as] generate strain new bacterium that resistant's is not effective in battling the disease. Nowadays, prevention and disease controlling become main priority in maintaining continuity of shrimp culture industry. Immunology of shrimp is a key element in determining strategy for disease control at shrimp culture. The usage of immunostimulants for example  $\beta$ -glucant and lipopolysaccharide (LPS) can improve nonspecifik immune response because it can improve phagocytosis activity from cellular defence, that have : effect on bactericidal activity In the other hand the shrimp will resistant to viruses diseses Using the immunostimulants in the shrimp culture for controlling the disease is a friendly environment because materials of the immunostimulants easy to reduce in the environments.

Table 3. The Average of Survival Rate (%) for Post Larvae of Tiger Shrimp Using Immunostimulants  $\beta$ -glucant and LPS after Challenged Test with White Spot Baculovirus (after grouping to Duncan Range Test)

| Treatments           | Average | Duncan Range Test |
|----------------------|---------|-------------------|
| Glucant 5 g/kg feed  | 9.670   | A                 |
| LPS 30 g/kg feed     | 6.840   | BA                |
| LPS 20 g/kg feed     | 6.630   | BA                |
| Glucant 10 g/kg feed | 6.170   | BA                |
| Glucant 15 g/kg feed | 4.340   | BA                |
| Control              | 0.710   | B                 |
| LPS 10 g/kg feed     | 0.710   | B                 |

Note: Treatment with the same character is not a significantly difference based on Duncan test

## ACUTE EXPOSURE OF THE NON TARGET ORGANISMS AT FUNGICIDE TERBINAFINE

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\*Fapesp: funding, proc. n° 2013/25113-2

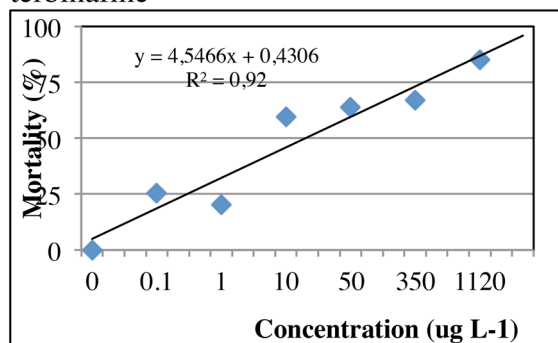
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Mycotic diseases in fish are often caused by *Saprolegnia* sp. oomycete which causing destruction of the skin and fins due cellular necrosis by hyphal penetration in fish. So, it is emergency the molecules study to control this pathogen but for molecules prospection is necessary to know the ecotoxicological effects for non-target organisms. Thus, the aim of this research was to evaluate the acute toxicity of terbinafine for fishes *Piaractus mesopotamicus* and *Hyphessobrycon eques* and for aquatic plants *Lemna minor*, *Azolla caroliniana* and *Wolffia* sp.

Fishes and plants were acclimated in the bioassay room at  $25 \pm 2^\circ\text{C}$  and initially, we performed sensitivity tests with reference substances for each organism. In the definitive toxicity tests, *P. mesopotamicus* was exposed to 20.0; 30.0; 45.0; 67.5; 101.2; 227.8; 341.7  $\text{mg L}^{-1}$ ; *H. eques* to 3.43; 11.15; 36.26 and 117.8  $\text{mg L}^{-1}$ ; *L. minor* to 0.1; 1.0; 10.0; 50.0; 350.0 and 1120  $\mu\text{g L}^{-1}$ ; *A. caroliniana* to 0.1; 1.0; 3.5; 11.2; 36.5 and 118.0  $\text{mg L}^{-1}$  and one control. The tests were conducted in static system, with three replicates where there were three fish, 12 fronds of *Lemna*, five plants of *Azolla* or plants in 0.3 mm of *Wolffia* per replicate. The fishes were exposed for 48 hours and the pants for seven days and in the end the  $\text{LC}_{50;48\text{h}}$  values were calculated by Trimmed Spearman-Kärber software. The  $\text{LC}_{50;48\text{h}}$  of terbinafine for *P. mesopotamicus* was  $58.96 \text{ mg L}^{-1}$  ( $41.69 - 83.38 \text{ mg L}^{-1}$ ); for *H. eques* was  $> 117.8 \text{ mg L}^{-1}$  because there was no mortality; for *L. minor*, the  $\text{LC}_{50;7\text{D}}$  was  $10 \text{ ug L}^{-1}$  ( $10 - 20 \text{ ug L}^{-1}$ ) (Figure 1); for *A. caroliniana*,  $4.35 \text{ mg L}^{-1}$  ( $3,10 - 6,11 \text{ mg L}^{-1}$ ) and for *Wolffia* sp. was  $> 118.0 \text{ mg L}^{-1}$ , because also there was no mortality of plants until this maximum concentration tested. The toxicity for *P. mesopotamicus* must be monitored in relation to exposure time because it is host of oomycete *Saprolegnia* which it will be controlled using terbinafine. Therefore, the best bioindicator for terbinafine toxicity is the plant *Lemna minor* because is more sensitive and classify this fungicide as extremely toxic ( $\text{LC}_{50} < 0.1 \text{ mg L}^{-1}$ ).

**Figure 1.** Relation of concentration and response of *Lemna minor* exposed at terbinafine



## INTESTINAL HISTOLOGY OF NILE TILAPIA FED DIETS CONTAINING PREBIOTICS, PROBIOTIC AND SYMBIOTICS\*

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\*Fapesp: funding, proc. n° 2014/17967-4

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The aim of this study was to evaluate histological changes of Nile tilapia intestine, *Oreochromis niloticus*, after supplement of prebiotic, probiotic and symbiotic feed during 63 days. The animals (mean weight=  $8.86 \pm 3.22$ g), were distributed in 40 L aquariums, in a density of 10 fish aquarium<sup>-1</sup>, with constant aeration and temperature of 26°C. The design was completely randomized with 6 treatments and 4 replications. The experimental rations were formulated to be isoprotein and isocaloric in two particle sizes: 1.0 and 2.0mm diameters.

The fish received six diets, as in Table 1.

In the diets P, S1 and S2 the bacteria addition was done after extrusion, to avoid impair the microorganisms. The commercial lyophilized PAS-TR® commercial probiotic containing *Bacillus cereus* and *B. subtilis* ( $4.0 \times 10^{11}$  FCU) was weighed in analytical balance ( $4\text{g kg}^{-1}$  of ration), homogenized in soybean oil ( $20\text{mL kg}^{-1}$  of ration). For better incorporation, the oil was spread on the ration. The same amount of oil was added to C, P1 and P2 diets. The rations were stored under refrigeration at 4 °C, and the fish were fed three times daily, *ad libitum*. At the end of the experiment, the animals were anesthetized and killed by deep sedation and spinal dissection. Different regions of the intestine were collected, fixed in Bouin solution and processed per conventional method for light microscopy. Cross sections were stained with toluidine blue + acidic fuchsin. The observations were for the preservation of enterocytes, dividing enterocytes (mitotic figures), amount of goblet cells, of blood vessels and of mast cells in the intestinal mucosa and submucosa. These changes were qualified in: - =not observed, + =little observed, ++ =moderately observed and +++ =much observed. In general, the C, P, S1 and S2 animals has presented relatively preserved enterocytes. At P1 these cells were better preserved and in P2 less preserved. Mitotic figures were not observed in any of the intestines evaluated. Goblet cells were observed in large amounts in all groups. Mast cells were mainly found at P animals. From these data, it was possible to conclude that intestinal cells were altered favoring the necessary protection of probiotic and symbiotics for the installation of undesirable bacteria.

Table 1- Nutritionals supply in feed for *O. niloticus* and concentrations used

| Diet                | Product   | Concentration<br>(g kg <sup>-1</sup> ) |
|---------------------|---|--|
| Control<br>(C)      | -----   | -----                                  |
| Prebiotic 1<br>(P1) | Actigen <sup>™</sup><br>(MOS)   | 4.0                                    |
| Prebiotic 2<br>(P2) | <i>Kappaphycus<br/>alvarezii</i>  | 4.0                                    |
| Probiotic<br>(P)    | PAS-TR®<br>( <i>Bacillus<br/>subtilis</i><br>$4 \times 10^8 \text{UFC g}^{-1}$<br>e <i>B. cereus</i><br>$4 \times 10^8 \text{UFC g}^{-1}$ ) | 0.4                                    |
| Symbiotic<br>1 (S1) | PAS-TR <sup>™</sup> +<br>Actigen®   | 0.4+ 4.0                               |
| Symbiotic<br>2 (S2) | PAS-TR® +<br><i>K. alvarezii</i>  | 0.4+ 4.0                               |



## CHARACTERIZATION OF IMMUNE-RELATED GENES IN SMALL YELLOW CROAKER (*Larimichthys polyactis*) USING NEXT-GENERATION DNA SEQUENCE ANALYSIS

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The small yellow croaker is a benthopelagic migratory fish species endemic to China and Korea; its habitats include, coastal and estuaries waters around the Yellow, East China and Bohai Seas. The small yellow croaker was once considered one of the top five commercially important marine fish species in China and Korea as food source and traditional medicine. However, the economic importance of small yellow croaker have led to its over exploitation to keep up with the consumer's demands, aquaculture farmers prefer culturing large yellow croaker than small yellow croaker due to the size difference. Some farmers have started culturing small yellow croaker but they still have a long way to go as the species has already been labelled as over exploited and studies about its management and conservation plans are still lacking.

The aim of this study was to use next-generation high throughput sequencing, bioinformatics, and comparative genomics to identify genes known to be associated with immunity in the genome of small yellow croaker (*Larimichthys polyactis*). In the absence of the small yellow croaker reference genome; the genome sequence of its sister species, large yellow croaker (*Larimichthys crocea*) was used as a reference for mapping of the next-generation sequence reads of small yellow croaker using bioinformatics tools. Five genes (*ccr5*, *Cd276*, *jak3*, *nod1* and *polr3e*) genes implicated with immune response in other teleost fish as well as in humans were annotated and predicated manually using prediction methods based on the homology and similarity search against NCBI nt and nr databases. The nucleotide and amino acid sequences were used to estimate the evolutionary relationship between the identified genes in small yellow croaker and their homologation from 7 teleost fish and humans.

Results exhibit revolutionary relationships between the immune genes in small yellow croaker and their homologs in other teleost fish species and divergence from their human homologs. However, *CD276* gene shows a closer relationship between the human homolog than some of the teleost fish homologs (*C. Carpool*, *S. salar* and *I. punctatus*). Multiple sequence alignment exhibits some level of conserved regions between species. The evolutionary relationship between the small yellow croaker and large yellow croaker is highly visible and they show that these two species share high genetic affinity. The role played by the identified genes in the immune system in fish is still lacking. Protein functionality for each gene was predicted using InterPro and GO web services; *ccr5*, *jak3* and *polr3e* genes are involved in biological process, molecular function and cellular components while *CD276* and *nod1* genes are only involved in biological process and molecular function, respectively. This study successfully identified genes involved in immunity in the genome of small yellow croaker; however, whether or not the immune system of small yellow croaker is well developed cannot be concluded. Additional studies on the genome of small yellow croaker are needed to assist in conservation and management plans and aquaculture of the species.

## EFFECT OF PROBIOTICS ON *VIBRIO* BACTERIAL POPULATION OF *Litopenaeus vannamei* IN CULTURE PONDS AT VADACHEEPURUPALLI, ANDHRA PRADESH, INDIA

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The current study was carried out for 147 days to assess the *Vibrio* count of *Litopenaeus vannamei* culture ponds from Vadacheepurupalli, Visakhapatnam District, Andhra Pradesh, India. Three ponds were selected, one is control and other two were experimental (Pond A and B). The physico-chemical parameters of the culture ponds were recorded by adopting standard methods. During summer crop the control ponds were harvested at 24.0 gm on 107<sup>th</sup> day and at 31.0 gm on 121<sup>st</sup> day for the year 2015 and 2016 respectively due to the incidence of *Vibriosis* disease. Where as in winter crop during 2015 the control pond was harvested at 5.5 gm on 47<sup>th</sup> day because of *Vibriosis* but in 2016 the control pond was harvested normally at 32.5 gm on 145<sup>th</sup> day, as this pond is free of *Vibriosis*. Experimental ponds were harvested normally in summer season at 30.5 gm on 124<sup>th</sup> day & 31.0 gm on 127<sup>th</sup> day during 2015 and 34.0 gm on 127<sup>th</sup> day & 35.5 gm on 127<sup>th</sup> day in the year 2016 respectively. Where as in the winter season of year 2015, experimental ponds were harvested normally at 28.0 gm on 124<sup>th</sup> day as well as on 127<sup>th</sup> day. In the year 2016 winter, the experimental ponds were harvested at 36.0 gm at 146<sup>th</sup> day and 35.5 gm at 147<sup>th</sup> day respectively. This study suggests that the probiotics are the key agents which have a great impact on the reduction of total *Vibrio* count in culture ponds of *L. vannamei*.

## COMMERCIAL AQUACULTURE FARMERS IN SOUTH AFRICA, ONLY AT THE AGE OF 15YEARS. YOUNG WOMEN IN ACTION TO MAKE A DIFFERENCE IN FOOD PRODUCTION AND SUSTAINABILITY

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In 2011 after watching the movie Dolphin tale I became interested in marine life, and soon realised that our marine resources are strained. I realized that waiting till I finish school and becoming a marine biologist might be too late: If I do not act now, there might not be any marine life left to work with.

Due to the fact that we live inland, I moved my focus to fresh water fish and making a difference there.

I started farming in January 2014, after I attended various courses that could help me gain the knowledge to be a successful fish farmer.

The first system consists two facilities. The main first facility consists of 4 x 7500 litre dams and a hatchery plus nursery with 15 x 60L fish tanks; a mechanical filter; a biological filter; a sump (gathering container for all the gravitating water in the system). Water total in the system adds to 38 000Liter.

The footprint covers 450m<sup>2</sup>. In total the system accommodates 18 000 fish. This is a ratio of 60kg fish/m<sup>3</sup>. I am over populating with 51.66% because the industry norm is 20/30kg/m<sup>3</sup>.

The overpopulation brought with it various challenges primarily relating to the quantity of dissolved toxins in the water. Water quality have a direct influence on the health of the fish, and I have to maintain good water quality to keep the mortality rate as low as possible.

After some research I concluded that adding aquaponics to the design will enhance the water quality. The plants will have the same effect as adding another biological filter 2.5 times the size of the current one in the design. The benefit that selling the plants had on an income opportunity, made the decision easy.

In the vortex filter all the solids descend to the bottom and drain from the system when the tap is opened. Previously the solids were discarded into the veld, but this resulted in a loss of between 1500 and 2000 litres water per day.

The lack of access to natural water rescores like a river or borehole make water an expensive and scares commodity. Wasting 2000l of water daily was no longer an option. An urgent solution had to be found.

I then, with the help of my dad installed a containment reservoir in a Decoupled Aquaponics System (DAPS). Bio solids from the mechanical filter are discarded to this reservoir on a daily basis. We erected a greenhouse tunnel to contain aquaponics and NFT systems. This aquaponics / NFT system is used as a purifier of the sludge / bio solids water contained in the reservoir. This system is a one loop aquaponics NFT system. Connected as a DASP. The purified water are reused to replenish the aquaculture facility due to loss of water in evapotranspiration and effluent discharge.

My second system consist of 10 x 4500 litre dams. The system will contain 20000 fish. Both systems will produce 10ton fish per year. The filter system that I'm using is an anaerobic and an aerobic filter. The anaerobic is TBX filter material. Each block is equal to a rugby field surface area. I'm making use of 15 blocks. My aerobic filter is also known as a fluidised filter, where aeration is pumped through a massif amount of filter media. The aeration helps with the growing of nitrifying bacteria. Back to a sump from where the pumps pump clean water to the fish dams.

I am privileged to have signed a contract to provide 1000kg of fish for the next 3 years.

## **FARMING TILAPIA (*Oreochromis mozambique's*) AND SHARPTOOTH CATFISH IN GAUTENG, SOUTH AFRICA BY A 15 YEAR OLD GIRL THE YOUNGEST COMMERCIAL FISH FARMER IN AFRICA, WITH A VISION TO FEED THE CONTINENT, IMPLEMENTING AQUAPONICS AND A DAPS SYSTEM TO SAVE WATER**

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### Problem or Question being addressed

A large amount of water is wasted on a daily basis a RAS system.

In a RAS Aquaculture system mechanical vortex filters are used to filter out heavy solids up to 200 microns. In the average facility waste water will be pumped into a secondary system like a river. This amounts to high volumes of water in small commercial plants of up to 3000 liters of water and big commercial plants up to 10 000 liters of water per day.

The system in this presentation has no access to natural water resources and adding water on a daily basis to maintain water levels results in high operation costs.

High level of bio solid sludge in fish dam water prevents constant recirculation.

How can loss of water be prevented?

How can the bio solid sludge water be purified for re-use in this aquatic system?

### Hypothesis

The successful re-use of Aquaculture waste water in an Aquaculture R.A.S. system. Reduces excessive water spillage/wastage, reduce high level of dissolved toxins, limits carbon footprint.

### Procedure to be followed:

Overstocking in Aquaculture facility with fish resulted into excessive excrete.

To eliminate excrete, mechanical vortex filters are used to drain all solid waste larger than 200 micron. To clean mechanical filters, huge amounts of water are discarded to flush heavy clogged solids from the system.

**Problem:** - all above mentioned water were discarded into the veld.

**Solution:** - direct all effluent water to a contained reservoir. All water is sent a contained reservoir.

Recirculate water that is contained in a reservoir through an aquaponic / hydroponic facility at a calculated flow rate and root density to clean the effluent water to a re-usable state and quality, to be used to refill the R.A.S. system.

In an Aquaculture framing environment regular water quality testes are mandatory. The same water tests will be done on the effluent system after circulation to test the water quality, and to indicate that by 8 hour circulation the results will be satisfactory that the water can be used to refill the fish dams.

### Prototypes build/used

Prototypes, drawings and analysis graphs including test results will be provided.

## PARENTAGE ANALYSIS AND GENETIC DIVERSITY IN A COMMERCIAL DUSKY KOB *Argyrosomus japonicus* POPULATION

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Dusky kob, *Argyrosomus japonicus*, is a Sciaenid finfish species, indigenous to South Africa. The species sustained both commercial and recreational fisheries as a high value commodity. Resultantly, the natural population has come under considerable pressure and is at risk of over-exploitation. In order to supply market demand, the species has been identified as an emergent aquaculture species in South Africa. Current production practices mostly rely on undomesticated (wild caught) broodstock to produce seed animals for the market. Mass spawning under commercial practices complicates the maintenance of pedigree records, which hinders the effective genetic management and implementation of breeding programmes, and could lead to lower production outputs in the long-term. The current study therefore utilised microsatellite markers to evaluate broodstock spawning success and contribution to commercial cohorts of dusky kob. The genetic properties of a wild population were compared to three temporal  $F_1$  cohorts that were sampled over a two-year period (*i.e.* from weaning to marketable size). Although there were no significant differences in estimates of genetic diversity between the  $F_1$  cohorts, the marketable-sized cohort experienced a significant increased relatedness. Despite a heterozygosity excess, likely a results of a genetic bottleneck, the  $F_1$  individuals displayed comparatively low levels of allelic diversity with respect to the wild population. Pairwise  $F_{st}$  estimates indicated significant genotypic differentiation between the wild and  $F_1$  individuals with a global  $F_{st}$  of 0.03 ( $P < 0.01$ ) detected. The  $F_1$  individuals was further characterised by a significant reduction in effective population size ( $\pm 6.9$ ). Parentage analyses indicated only 7 full-sib groups, with one male siring approximately 89% of the  $F_1$  individuals. These results indicate large discrepancies in overall broodstock contributions to seed production. This has implications for the effective management and implementation of selective breeding programmes. Continued monitoring is therefore advised.

## EFFECT OF CRUDE OIL EXPOSURE ON MATURATION AND SPAWNING IN CAPTIVE FLORIDA POMPANO *Trachinotus carolinus* BROODSTOCK

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A number of studies are being conducted to assess the long term impact of the 2010 *Deepwater Horizon* oil spill in the Gulf of Mexico (GOM). Research demonstrates that oiling caused a wide range of biological effects including those on GOM fisheries populations. While much of the research has focused on assessing the effects of the spill on larval fishes, certain aspects are still understudied, such as those examining the physiological response of adult marine fish species.

In the present study, selected reproductive parameters were examined in captive Florida pompano broodstock following aqueous exposure to crude oil. A mixture of oil and the dispersant (Corexit, 9500) was used to produce chemically-enhanced water accommodated fractions (CEWAFs) (0.0, 0.5 and 1.0 ppm [corresponding to control, low and high treatments, respectively]) administered over 12-hours in a spiked declining exposure study. This was followed by a period of non-exposure for 24 hours and then the process was repeated. Tagged fish, 5 females and 10 males per treatment, were transferred from the exposure tanks to a closed recirculating aquaculture system (total volume 28m<sup>3</sup>) where they were maintained for five months under simulated natural (27 ± 1.0°C and 13.5hr of light) photo-thermal conditions. Female and male reproduction condition was assessed periodically throughout the trial. Ovarian biopsies obtained from individual females were prepped as wet mounts and for histology. When possible, milt samples were collected; sperm density, sperm motility and spermatocrit were documented among individual males. Blood samples were collected and the plasma steroids Testosterone and 11-ketotestosterone were analyzed. Mature females were implanted with Ovaplant® (GnRHa) at a dosage of 50 µg kg<sup>-1</sup> bw and placed into individual spawning tanks along with two males from the same treatment group. Over the course of the study, spawns were obtained from fish in all treatment groups. Total egg production for each respective treatment group were as follows: 169,000 to 237,000 (control), 47,000 to 200,000 (low), and 58,000 to 162,000 (high). Effects of oil exposure on reproduction and spawning performance in male and female Florida pompano will be discussed.

## DIETARY PROTEIN TYPE INDUCES DIFERENT INTESTINAL RESPONSE IN MEAGRE, *Argyrosomus regius* JUVENILES

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Previous studies reported that meagre is able to grow on dietary protein obtained from alternative ingredients to fish meal. This study was aimed at characterizing intestinal functionality *in vitro* using an Ussing-like chamber, in meagre juveniles fed diets with different protein content and when fish are fed diets with different protein source ingredients.

Meagre juveniles ( $n=80/\text{tank}$ ;  $52\pm0.6$  g) were fed for 12 weeks with two diets with the same ingredients but differing in the protein content (A - 52% and B - 46%) and a third diet (C) with 46% level of protein but that differed in ingredients (blood meal, feather meal and soya). Experimental diets in triplicate. At the end of the trial 12 fish from each treatment were sedated with of anesthetic, sacrificed and anterior intestine collected. Intestine was opened and mounted on Ussing chamber. Preparations were followed every 30 min until 120 min. Basal measurements of bioelectrical variables for short-circuit current ( $I_{sc}$ ,  $\mu\text{A cm}^{-2}$ ) and epithelial resistance ( $R_t$ ) in tissues voltage-clamped (to 0 mV), and bathed with the same saline solution. Statistical comparisons were pair-made for protein level (A& B) and type of ingredients (B& C).

Meagre specific growth rate (SGR,  $\%.\text{day}^{-1}$ ) was similar despite the different dietary content ( $P>0.05$ ; A -  $1.10\pm0.09$  & B -  $1.03\pm0.11$ ). However type of ingredients affected meagre SGR when equal amount of protein was used ( $P<0.05$ ; B & C -  $0.881\pm0.11$ ). Similar trend was observed for bioelectrical parameters, since  $R_t$  was similar despite the protein content, but differences were observed when ingredients were altered. This was more notorious for the current, indicating that fish fed diet C seem to allocate more energy to intestinal transport of nutrients.

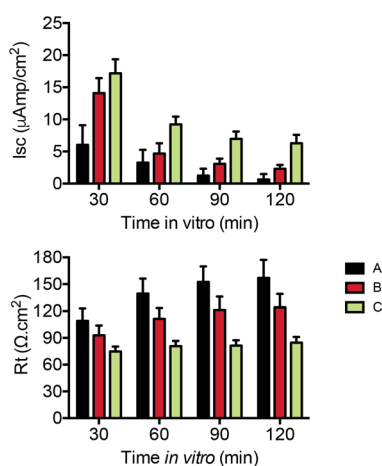


Figure – Bioelectrical variables determined on meagre intestine.

**Acknowledgments:** Research funded by project DIVERSIAQUA (MAR2020, Portugal) and PTDC/MAR-BIO/3034/2014 to J.F. CCMar is supported by national funds from the Portuguese Foundation for Science and Technology (FCT) through project UID/Multi/04326/2013.



## IMPROVEMENTS OF REPRODUCTIVE PERFORMANCE OF THE WHITE SHRIMP *Litopenaeus vannamei* BY ENRICHING BROODSTOCK DIET WITH *Ulva clathrata*: COMPARISON IN TWO COMMERCIAL HATCHERIES

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The effect of supplementing dehydrated *Ulva clathrata* in fresh diets on the reproductive performance of *Litopenaeus vannamei* broodstock was evaluated in two commercial hatcheries: one located in La Paz, Baja California Sur (hatchery A) and the second in Mazatlan, Sinaloa (hatchery B). Although *Ulva* powder was added in same way in both hatcheries (20g per kg of the thawed squid portion), management conditions differed between hatcheries in many aspects; one used eyestalk ablation while the other did not, the female size was different, the photoperiod differed, the number of organisms used per tank, the spawning conditions, and broodstock genetics. The daily mortality was significantly higher ( $P<0.01$ ) for females in the control group of hatchery A (ablated females). No differences in mortality were found for females in hatchery B, or in males from either hatchery. The frequency of daily molts was not significantly different between treatments in either commercial hatchery. In hatchery A, broodstock fed *Ulva* supplement produced more eggs (+5% increase compared to control,  $P<0.01$ ) and nauplii (+18%,  $P<0.001$ ) per female and had an increased hatching rate (+13%,  $P<0.001$ ). The number of nauplii production per day had also a tendency to be higher for the broodstock fed *Ulva* (+24%,  $P=0.061$ ) compared to control diet. In hatchery B, broodstock fed *Ulva* produced significantly more eggs (+26%) and nauplii (+33%) per day, and the hatching rate also increased (+7%). The number of spawns per day had a tendency to be higher (+20%,  $P=0.067$ ) for broodstock fed *Ulva*. We conclude that supplementation of *Litopenaeus vannamei* broodstock's fresh diets with the dehydrated seaweed *Ulva clathrata* enhanced broodstock reproductive performance at commercial scale regardless of the management conditions.

# SUPPLEMENTATION OF BROODSTOCK DIETS WITH *Ulva clathrata* IMPROVES REPRODUCTIVE PERFORMANCE AND OVARY NUTRIENTS ACCUMULATION OF *Litopenaeus vannamei*

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The maturation feed most commonly used nowadays in commercial shrimp hatcheries is composed of fresh frozen invertebrates (squid, polychaete worms, mussels, krill, *Artemia* brine shrimp, etc.), which provide yields that are generally good but can still be improved (1, 2). Unfortunately, fresh ingredients seasonal variations, conservation and process deficiencies, or inadequate distribution limits the quality of nutrients provided to broodstock, and nutrient deficiencies might occur, that can curtail the development and reserve accumulation in the gonad. In the present study, the effect of supplementing broodstock fresh diet with dehydrated *Ulva clathrata* (0.8%) were assessed on the reproductive performance of *L. vannamei* and on its ovary biochemical composition (protein, carbohydrates, total lipids, triglycerides carotenoids, and total phenolic contents (TPC)) as well as antioxidant activity (DPPH) at different development stages. Individually tagged eyestalk-ablated females production of nauplii and eggs was monitored for 45 days in a commercial hatchery; females of different maturation stages were sampled at the end of this period to analyze their ovary composition. The females fed the *Ulva clathrata*-enriched diet had significantly higher egg production (+5%), hatching rate (+13%), and nauplii production (+18%). They also presented bigger oocyte area per stage and higher levels of total lipids, triglycerides TPC and DPPH in gonads. The supplementation of the maturation diet with 0.8% dried *Ulva clathrata* is therefore an efficient innovative strategy to enhance the broodstock reproductive performance.

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## MICROBIAL – DEPOSIT FEEDER AQUACULTURE BIOREMEDIATION SYSTEMS

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Land-based intensive aquaculture produces large volumes of particulate organic waste that can be upcycled into high value secondary biomass. In this research, the application of two key principles underpinning low-cost bioremediation technologies, namely the addition of rate limiting (i) electron acceptors (oxygen), and (ii) donors (carbon) is investigated in a sediment-based aquaculture effluent treatment system integrating the sea cucumber, *Holothuria scabra*. Growth trials of *H. scabra*, combined with next generation sequencing (NGS) technologies, were used to examine the response of sea cucumbers and sediment bacterial communities under contrasting redox regimes, describing fully oxic and redox-stratified sediments. The oxic system resulted in high taxonomic and functional diversity of bacteria with a range of dissimilatory metabolisms required for successful bioremediation of aquaculture wastes; however, the final biomass of *H. scabra* was significantly lower than the redox-stratified sediments ( $449.22 \pm 14.24 \text{ g m}^{-2}$  versus  $626.89 \pm 35.44 \text{ g m}^{-2}$ ). Improving the resource quality of aquaculture waste through carbon supplementation was investigated. Increasing the carbon/nitrogen ratio from 5:1 to 20:1 with soluble starch significantly increased the biomass production of *H. scabra* compared to controls ( $1011.46 \pm 75.58 \text{ g m}^{-2}$  versus  $702.12 \pm 35.93 \text{ g m}^{-2}$ ). A benthic flux incubation study, combined with NGS, demonstrated that carbon supplementation did not change the pathway of nitrogen cycling by mediating a shift from net release of ammonium to net assimilation, as hypothesised. A final study elucidated the critical role of the sea cucumber microbiome during aquaculture waste bioremediation, demonstrating that endogenous bacteria are primed, at ecological and genomic levels, to respond to nitrogen - a key nutrient limiting deposit feeder growth. Deposit feeder-microbial aquaculture bioremediation systems have the potential to rectify current inefficiencies of nitrogen use in the aquaculture production chain by offering a more economically and environmentally sustainable alternative to closing the nitrogen cycle loop.

## THE MICROBIOME IS THE NEXUS BETWEEN SEDIMENT BIOREMEDIATION AND DEPOSIT FEEDER GROWTH

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Deposit feeding species are proposed as candidates for effluent bioremediation within aquaculture production systems. We evaluated the role of the system microbiome in regulating growth efficacy of *Holothuria scabra*, a commercially important sea cucumber (Holothuriidae), cultured on either organically enriched shrimp pond sediments or organically poor calcareous dune sand when fed isonitrogenous finfish particulate waste or a commercial formulated feed.

DNA was extracted from the following “feeding chain” sites: pre-ingestion sediments, digestive system (foregut, midgut, hindgut), and faeces, in response to sediment and feed type from replicated treatments. Amplicons of the V4 region of the 16S rRNA gene were sequenced on an Illumina MiSeq. Bacterial community composition and environmental drivers were analysed by canonical correspondence analysis. Functional metagenome predictions were made using Tax4Fun.

Feed and sediment affected holothurian growth over the 140 day trial, with dune sand and formulated feed treatments outperforming all others. Sampling location along the sea cucumber feeding chain had a significant effect on all alpha diversity metrics. Bacterial community richness and diversity was highest in the ambient sediments prior to ingestion and decreased with passage through the gut to reach the lowest diversity in the midgut before progressively increasing in the hindgut and faecal mounds. The bacterial community composition was dominated by Proteobacteria, Bacteroidetes, Firmicutes and Planctomycetes.

The presence of a unique bacterial community in the midgut of *H. scabra* was detected and characterised. The core midgut community was taxonomically and metabolically diverse and exhibited an enhanced capacity to respond to increasing nitrogen availability, a key nutrient commonly considered to limit deposit feeder growth by increasing purine and nitrogen metabolism. The significant increase in amino acid metabolism and metabolism of vitamins and co-factors in the hindgut of sea cucumbers reared on shrimp pond sediments supports the hypothesis that the gut microbiome plays a key role in nutrient provisioning to *H. scabra*.

The study highlighted the important role that endogenous bacteria play in nitrogen metabolism and the remediation of aquaculture wastes. Understanding these interactions will inform the effective design of aquaculture effluent treatment systems that optimise deposit feeder growth while concurrently providing effective waste remediation.

**EARTHWORM WASTES USED TO ENHANCE PRODUCTIVITY IN A WHITE SHRIMP *Litopenaeus vannamei* EXPERIMENTAL CULTURE**

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In Mexico the annual shrimp aquaculture rate production is growing, and thus demands more feeding inputs, representing ~50% of the operative costs (CONAPESCA, 2011). In shrimp culture, fertilization is needed to improve primary productivity (Clifford, 1997). Among organic fertilizers, *Eisenia foetida* humus is considered as a great promoter for plankton growth, helping to develop these aquatic organisms (Chakrabarty, 2008). The aim of this study was to analyze and evaluate different earthworm humus as phytoplankton promoters. Six treatments were evaluated, in 12 L tanks with salt water (10 ups), zero water exchange and the following initial doses: control, Urea 0.45g·L<sup>-1</sup>, solid humus- cattle manure worm compost[WC] 0.5g·L<sup>-1</sup>, T4 liquid humus- cattle WC 0.1L·L<sup>-1</sup>, T5 solid humus- vegetable wastes WC 0.5g·L<sup>-1</sup> and liquid humus- vegetable wastes WC 0.1L·L<sup>-1</sup>, with three replicates for each one. The doses were, thereafter, reduced by half. The experiment lasted 33 days. Samples were quantified and identified to group level.

Water quality parameters were not constant during the experiment and had significant difference (P>0.05) between oxygen and pH treatments. For phytoplankton quantity, treatment with humus had the highest number of cells with 551, 500 cell ml<sup>-1</sup>, but had no significant difference with Urea who had 500, 650 cell ml<sup>-1</sup> (Figure 1). It had significant differences with other treatments. Four different phytoplankton groups: diatoms, chlorophytes, cyanophytes and nanoplankton, were identified. We found that shrimp reared with the organic fertilizer had the best survival (Table 1), It had significant differences with other treatments. Four different phytoplankton groups: diatoms, chlorophytes, cyanophytes and nanoplankton, were identified. We found that shrimp reared with the organic fertilizer had the best survival (Table 1), but the lowest final weight. Some studies suggest that diatoms and chlorophytes are an important food resource for juvenile shrimp (Chakrabarty, 2008). Organic fertilizers improved water quality and enhanced phytoplankton production; we observed that none identified nanoplankton had significant differences with diatoms, chlorophytes and cyanophytes (Bwala and to enhance shrimp survival and increase the food production. The use of vermicompost as a nutrient promoter, in any of its forms, (solid and liquid) could be an inexpensive option as an organic fertilizer, in the shrimp aquaculture industry as well as for other species.

Figure1. Number of Cells by week during the experiment.

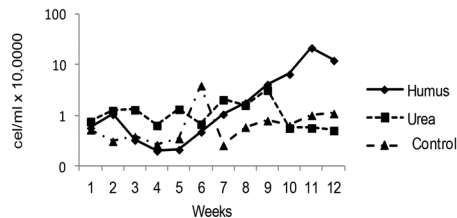


Table 1. Growth, final weight and survival of White shrimp *litopenaeus vannamei* with three different fertilization systems.

|         | Growth    | Final weight            | Survival                 |
|---------|-----------|-------------------------|--------------------------|
| Humus   | 0.09±0.03 | 6.89±2.10 <sup>a</sup>  | 92±12.12 <sup>a</sup>    |
| Urea    | 0.13±0.01 | 10.12±1.63 <sup>b</sup> | 83±8.19 <sup>a</sup>     |
| Control | 0.11±0.03 | 8.52±2.26 <sup>c</sup>  | 81.67±15.31 <sup>a</sup> |

## NUTRA LIFESTART FOR TILAPIA - A GLOBAL TEAM APPROACH TO TILAPIA STARTER DIETS

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The global tilapia industry sector is developing rapidly and Skretting is producing tilapia feeds in Africa, Americas, Asia and Europe. Our global tilapia team of product managers identified the need for a dedicated tilapia starter feed.

A wide range of starter feeds and feeding practices exists for tilapia around the world. These starter feeds are seldom formulated for tilapia but rather for a multispecies approach. Technology is often simple and single ‘powders’ are fed to tilapia larvae from hatch till fingerling. Therefore, tilapia fry do not get the right nutrition offered in too small and unstable particles which does not contribute to a good lifestart.

We commissioned Skretting Aquaculture Research Centre to develop a dedicated tilapia starter feed with the following specifications:

- Establish the protein requirements of tilapia larvae, fry and fingerlings, respectively
- Establish the energy requirement of tilapia fry
- Compare the latest feed technology in extrusion and micropelleting.

Research results will be presented and discussed (see for example Figure 1 as an illustration). Based on the findings a dedicated tilapia starter feed has been defined in 4 sizes:

- Nutra 0 - 400 micron extruded crumble for the larval and sex-reversal phase
- Nutra 80 - 800 micron extruded crumble for fry from 0.5-2.5 grams
- Nutra 120 - 1200 micropellet for fry from 2 - 10 grams
- Nutra 160 - 1600 micropellet for fry from 8-25 grams

Optimal early lifestart nutrition for tilapia is the fundament for profitable tilapia farming.

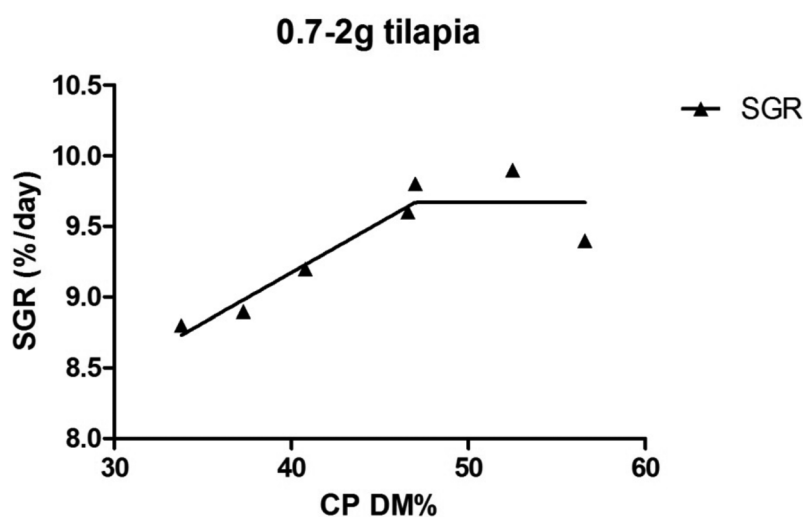


Figure 1: Broken-line plot of Specific Growth Rate and dietary protein for tilapia fry.

## EVOLVING APPROACH TO BIOSECURITY IN THE U.S. POULTRY AND LIVESTOCK INDUSTRIES

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Highly pathogenic avian influenza (HPAI) H5N2 affected over 200 poultry premises in the upper Midwest of the U.S.A. in spring 2015 and caused the destruction of nearly 50 million birds. The scope of the outbreak demonstrated that biosecurity practices for poultry facilities needed to be strengthened to reduce the risk of future outbreaks. To assist poultry producers in implementing stronger biosecurity plans, the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) worked with State, academic, and industry experts to develop a biosecurity self-assessment checklist and a Poultry Biosecurity Officer Information Manual.

The biosecurity approach before the HPAI outbreak typically focused on individual steps to prevent disease introduction (e.g. footwear disinfection, etc.). The new approach identified all potential routes of disease introduction and attempted to mitigate those routes of introduction. The enhanced approach to biosecurity emphasized three concepts that were new to most biosecurity plans:

- A Biosecurity manager who is responsible for developing a site-specific biosecurity plan, training all personnel who enter the farm, and monitoring for compliance.
- A Perimeter Buffer Area around buildings or pens which house animals and functions to reduce the infectious load near the buildings/pens.
- A Line of Separation which functions to provide a boundary between the susceptible animals and all potential sources of infection. This is easiest to implement for animals housed totally in buildings, but this concept also helps to protect animals housed outside.

A 12 point checklist covering all potential sources of disease introduction was developed along with a Biosecurity Manager Information Manual intended to help the Biosecurity Manager implement and maintain the site specific biosecurity plan. The National Poultry Improvement Program (NPIP) has adopted a modified version of this biosecurity plan and will require that poultry producers who are part of the voluntary NPIP follow the biosecurity checklist and are periodically audited for compliance. Online training materials for employees were also developed. All materials can be found here: <http://www.poultrybiosecurity.org/>

The same concepts have since been recommended for enhanced biosecurity for the swine, beef, and dairy industries to help protect production sites from foot and mouth disease virus in the event of an outbreak.



## DETECTION OF *Anisakis simplex* PROTEINS IN FISH PRODUCTS

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*Anisakis spp.* occurs in a wide range of hosts involving fish and marine mammals. The environment in which this parasitic worm is present are oceans and seas across the globe. Marine fish, mammals and crustaceans are the most common hosts for *Anisakis spp.* Anisakiasis is a zoonosis caused by the ingestion of living larvae present in raw seafood. The symptoms of this disease include abdominal pain, vomiting, nausea and diarrhea. Diagnosis of anisakiasis is mainly based on endoscopy. In most cases the disease can be resolved with only symptomatic treatment, however in some cases a surgery removal of nematode is necessary, especially in case of ulcerosis and necrosis of stomach wall. Moreover, *Anisakis* larvae release a set of proteins into the surrounding tissues when they infect a fish. Consumption of contaminated tissue may lead to acute allergic manifestations, such as urticaria and anaphylaxis. The aim of the project was to develop the system of methods for detection of parasitic nematodes proteins in fishery products. Two fast and reliable methods were elaborated - the sandwich ELISA and dot blot for the detection of *Anisakis spp.* proteins. Both methods allow for detection of one larvae crude extract in 200g fishery product, raw and heat processed. Selectivity was confirmed against the *Ascaris sp.* and *Toxocara sp.* larvae. No cross reaction (Fig.1) was observed. Effect of matrices was examined with use of aquaculture fresh water trout (*Salvelinus fontinalis*). No cross reaction to different matrices was observed. Also, the effect of heat treatment was examined. The decrease of OD value up to 18% was observed in case of heat treated samples compare to unheated once, but still we were able to detect proteins obtained from one larvae in 200g sample (Fig.1). The same results were obtained with the dot blot assay (Fig. 2). The developed system of methods allows the complete examination of fish and fishery products for the presence of *Anisakis* allergens. As the result of the study guideline for parasitological examination of fish for the presence of *Anisakis* allergens was elaborated. Research was supported by NCBiR under the Strategic Program Biostrateg (grant no. 296211/4/NCBR/2016).

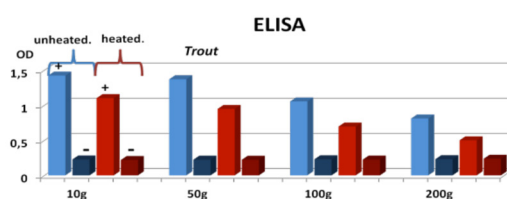


Fig.1 Effect of heat treatment on ELISA results

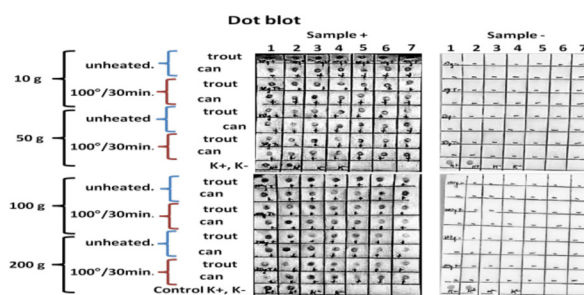


Fig.2. Dot blot results - the effect of matrices and heat treatment

## DETECTION OF ANISAKIDS IN BALTIC FISH AND FISHERY PRODUCTS - PRELIMINARY RESULTS

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In the last decades public health authorities have detected a relevant increase of seafood-borne diseases. The most hazardous helminths for human health, originated from marine fish, are anisakid nematodes. Human diseases are either caused by ingestion of viable parasites, or as an allergenic reaction. The symptoms of this disease include abdominal pain, vomiting, nausea and diarrhea. Diagnosis of anisakidosis is mainly based on endoscopy. In case of ulcerosis and necrosis of stomach wall surgical excision may be necessary. As there is a lack of sufficient data on the geographical distribution, prevalence and intensity of parasites of public health importance in fishery products, all wild-catch products are considered at risk of containing parasites. Parasites can affect the commercial value of fish and thus result in significant economic losses. Fish caught from fishing grounds in the Baltic Sea, may present possible health risks from: *Anisakis simplex* (*sensu stricto*), *Contracaecum osculatum* (*sensu stricto*), *Pseudoterranova decipiens* (*sensu stricto*). The *A. simplex* and *Pseudoterranova decipiens* have been found in fishery products in International Council for the Exploration of the Sea (ICES) subdivisions 22, 23, 24, 25, 26, thus the public health risks due to the presence of these parasites cannot be excluded. Part of the SeaQual project is devoted to assess the presence of Anisakids in fish from Baltic Sea. The aim of the carried study is to collect the data on the presence of parasites in wild caught fish from the Baltic Sea for further risk assessment. For the study herring (*Clupea herrengus*) and cod (*Gadus morhua*) caught in the Baltic Sea were examined. Samples were collected from the sea catch provided in April 2016 by the research vessel “Baltica” (fig.1). Totally 246 herring and 228 cod samples were collected. Fish samples were examined by candling method, candling with UV and digestion of muscle tissue (fig.2). All the methods were validated; candling and digestion were accredited by the Polish Centre for Accreditation. Overall, 14.23% of herring and 9.65% of cod were found to be positive with the use of digestion method. The prevalence in herrings varied from 2.35% (ICES 25) up to, 23% (ICES 26). For cod varied from 6% (ICES 24) up to 15% (ICES 25). From cod muscle as *Anisakis simplex* s.s. 20 larvae were identified and 2 larvae were classified as *P. decipiens*. Larvae originated from herring were identified as *Anisakis simplex* s.s. in 30 cases, and 5 larvae were classified as *P. decipiens*. Species of anisakids were identified by PCR-RFLP. As the result of the study guideline for parasitological examination of fish for Veterinary Service was elaborated.

Research was supported by NCBiR under the Strategic Program Biostrateg (grant no.296211/4/NCBR/2016)

## PERFORMANCE AND PROFITABILITY OF AN INTEGRATED AGRICULTURE AQUACULTURE SYSTEM: RABBIT-FISH-RICE SYSTEM IN RWANDA

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Integrated farming is a costly activity that hinders initiatives of rural farmers until they are confident of possible returns and/or profitability.

This study was carried out to evaluate the growth and yield performance and the profitability of rabbits, Nile tilapia, and rice paddy under a rabbit–fish–rice integrated (IRFR) system in tropical semi-intensive farming, and assess the extend integrated aquaculture should benefit to a resource poor fish farmer. The experiment allowed rearing of rabbits over six ponds, three of which were stocked with one fish per m<sup>2</sup> and three with three fish per m<sup>2</sup>, and nine rice fields, of which three received inorganic fertilizers and six received fish ponds effluents. Results revealed that rabbits performed with 89% survival and 57% increase of individual weight in 101 days. *Oreochromis niloticus* yield was dependent on stocking density, and 1.94 t.ha<sup>-1</sup> fish gross yield were obtained with 3 fish.m<sup>-2</sup> stocking rate after 112 days without supplemental feed. Effluents led to higher rice yields in IRFR (5.87 t.ha<sup>-1</sup>.crop<sup>-1</sup>) that were not significantly different from yield (5.79 t.ha<sup>-1</sup>.crop<sup>-1</sup>) in rice fertilized inorganically. The IRFR system increased the net return up to 596.9% (US\$30 – 208) over that of rice monoculture and was fish density dependent.

The IRFR system works well, is readily applicable, and can be promoted for optimum resources use, income generation, and environmentally friendly productions.

## OYSTER FARMING IN NAMIBIA: A SUCCESS STORY TO GREATER FOOD SECURITY

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Namibian mariculture is currently expanding significantly, primarily because international markets for oysters are being opened up, particularly in Asia. Up till now Namibia's main mariculture species has been Pacific oysters, and its main market South Africa. Only having one key market stifled production and Namibia's mariculture industry remained small until recently. The industry started making international buyer contacts in South East Asia, through business trips, as a foundation for starting their production operations around the beginning of 2006. They obtained such large international orders that several companies had to cooperate in an attempt to meet demand. There is a real opportunity to expand production for other Namibian oyster companies by establishing good market contacts internationally. This involves market research, visits to international trade shows, and visits to international clients to cement relationships and meet client product specification requirements.

Most products at the moment are shipped live. Oysters, fresh in the half-shell are sold to cruise liners when they come alongside at the Port of Walvis Bay. There is some direct selling by growers to restaurants, but this involves a lot of work for small orders, so generally it is easier to sell to Namibian wholesalers. Some sales also occur to supermarkets and local tour operators. Whole frozen oysters are currently sold to the distributor Blue Marine Interfish, based in Windhoek. They distribute them domestically and in the SADC market, where there is good demand for this product. The Namibian market for oysters is very small, but it can be expanded through more aggressive marketing and by greater availability. To some extent, it can also be promoted through tourism. If we assume that that present domestic oyster consumption is approx. 30 tonnes per year, and we assume that marketing initiatives are made, we estimate that future domestic consumption will be 50 – 60 tonnes per year.

The international market for oysters may appear to be huge. Global production of oysters is presently more than 4.5 million tonnes, of which most is produced and consumed in China. However, only about 50,000 tonnes enter the international markets annually. In 2005, total global export trade in oysters amounted to some 50,000 tonnes worth US\$ 166 million. In other words, most of the oysters produced in for example China appears to be consumed domestically. During the period from 1992 through 1997 there was stagnation in international trade, and unit prices fell.

Pacific oysters are sold regionally to South Africa as a live product. Currently efforts to open overseas markets for live, fresh and frozen oysters to Hong Kong, Beijing and Singapore as well as the USA are being made. Singapore acts as a logistical trade hub for onward sale by Singapore distributors into other parts of Asia. Europe and the USA are expected to open up as markets when the Namibian Shellfish Sanitation Programme is operational and EU accredited. The bulk has traditionally been sold to wholesalers in Johannesburg, and smaller amounts to wholesalers in Cape Town, who then sell to restaurants and hotels. Some product is also sold to oyster farms in South Africa for on growing. Present indications from markets in South East Asia are that this market would be able and willing to absorb the entire production in Namibia, even at a production volume of 3,000 – 4,000 tonnes a year.

Based on FAO figures, Namibian oyster production currently amount to less than 0.01% of world production. Consequently, if they become popular overseas, demand will well outstrip supply. A market strategy needs to be devised to maximize the value of Namibia's oysters, and to expand production within the context of niche marketing, selling to high-end buyers who are willing to pay a premium for the product. A goal could be to make Namibian oysters "the most expensive oysters in the world". It is clear from a „value" perspective, and given the taste and texture of Namibian oysters, that the opportunity for Namibian oyster growers is with whole „live", and fresh or frozen half shell oysters. These are the higher value segments, and are considerably more expensive than the high volume canned and processed forms such as those that come from Korea. A good way to introduce a product at the high-end of the market is via expensive hotels and restaurants. Many countries have learnt the success formula of promotions through top hotels and restaurants. More broadly, a „total industry" commitment to an agreed set of verifiable standards that could be communicated via joint marketing efforts should be considered by the mariculture sector.

*(Continued on next page)*

For example, the industry could agree to implement „best practice“ quality control systems together with environmental and resource sustainable farming and harvesting practices that would enable the industry to clearly position itself as a „world class“ provider of oysters. A „total industry“ approach, strongly aligned to Namibia’s “pristine, naturally productive waters”, will help establish true points of difference with the major oyster buyers Namibia is targeting. This strategy only calls for Namibian oyster exporters to promote, showcase and market in a collective and consistent manner. What is important is to establish and market the „marketable advantages“ that come with a joint marketing strategy, to ensure that Namibian oyster exporters are able to negotiate from a position of quality, consistency, environmental sustainability, and food safety. This strategy is much more than just jointly funding display space at seafood shows and developing glossy generic brochures. Undertaken properly, it will create points of difference for Namibian oysters in the international arena.



## EFFECT OF DIETS WITH DIFFERENT HISTIDINE LEVELS ON MEAGRE *Argyrosomus regius* LARVAL SURVIVAL, GROWTH AND MUSCLE CELLULARITY

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Deficient dietary histidine levels have resulted in poor growth rates and lower food conversion rates in several fish species. Histidine is an indispensable amino acid and has an important role in protein synthesis.

Muscle growth occurs by two different processes: muscle fibre hypertrophy, which is the increased of already existent fibres and muscle fibre hyperplasia which consists on the recruitment of new fibres. Some studies showed that different histidine levels affected the percentage of hypertrophy and hyperplasia. The aim of this study was to evaluate the effect of diets with different histidine percentages on meagre larval survival, growth and muscle cellularity.

Three diets were tested in triplicates in 18 days after hatched larvae: a control diet with a balanced AA profile (based on Saavedra et al., 2015), a diet with 1% histidine supplement (HIS 1%) and a diet with 3 % histidine supplement (HIS 3%). HIS 1% and HIS 3% had the same formulation as the control diet. Larvae were reared in 200 l tanks and fed the inert diets in cofeeding with *Artemia* until they were 28 DAH. From 29 DAH until the end of the trial (38 DAH) they were only fed the inert diet. At the end of the trial survival and growth were quantified. Muscle cellularity was analysed in the region of the first dorsal ray by histology.

The results showed that survival and growth were not significantly affected by an increase of dietary histidine but larvae fed the HIS 1% diet showed a higher final length compared to the control. The analysis of muscle cellularity showed that hyperplasia was the most important growth process as more than 50 % of fibre area was lower than 150  $\mu\text{m}^2$  in all treatments. Mean fibre area was  $172 \pm 53 \mu\text{m}^2$ ,  $149 \pm 51 \mu\text{m}^2$  and  $187 \pm 47 \mu\text{m}^2$  for control, HIS 1% and HIS 3 %, respectively.

The results obtained in this study suggest that the AA balanced diet, based on the AA requirements obtained by Saavedra et al. (2015) does not affect significantly meagre larval survival and muscle cellularity.

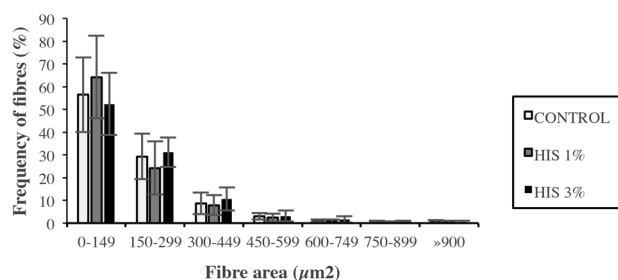


Fig. 1. Frequency of fibre areas in 38 days after hatched meagre fed a control diet, a 1% histidine (HIS 1%) and 3 % histidine (HIS 3%) supplemented diets.

## EFFECT OF DIFFERENT WATER TEMPERATURES DURING MEAGRE LARVAL ONTOGENY ON MUSCLE FIBRE CELLULARITY IN LATER FISH DEVELOPMENT STAGES

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Understanding how muscle growth occurs and the factors involved in it is of extremely importance not only to optimize fish growth but, as well, to achieve a better flesh quality as texture is an important quality trait, often associated to muscle structure. The aim of this study was to evaluate the impact of using two different temperatures during larval stages on fish muscle cellularity in later stages.

Newly hatched larvae were distributed into 120 l parallelepiped tanks at a density of 50 larvae/ l. Tanks were randomly distributed in two different water temperature treatments: three under natural temperature and three with an increased water temperature ( $21.5 \pm 0.9$  °C and  $22.9 \pm 0.6$  °C, respectively). When larvae were 30 days old, they were transferred to 1500 L tanks, all at natural water temperature. Fish growth and muscle cellularity were analysed at 1, 2, 4, 6 and 12 months. Muscle cellularity was analysed in the region of the first dorsal ray by histology.

Results showed that 1 month larvae under the HT treatment had a higher dry weight compared to control and that most fibre areas were distributed between 2 and  $150 \mu\text{m}^2$ . In the control treatment 50 % of the fibres were smaller than  $50 \mu\text{m}^2$ . In 2 months old meagre, the percentage of newly recruited fibres ( $<150 \mu\text{m}^2$ ) was still high, above 50%. This percentage decreased substantially in 4 and 6 months old meagre and was reduced to nearly 10 % in 12 months old fish. Most differences between treatments were found when fish were 4 months old. Larvae subjected of higher water temperature showed higher percentage of newly recruited fibres. On the contrary, larvae subjected to the natural water temperature showed higher percentage of larger fibres (from 1200 to  $1799 \mu\text{m}^2$ ). The differences in the frequency of hyperplasia and hypertrophy, observed in 4 month fish, may have been responsible for a higher growth rate of fish from the control group as both groups, from that point on, showed similar biometry.

This study shows that water temperature affects both growth and muscle cellularity of meagre larvae as well as muscle growth pattern in later developmental stages.

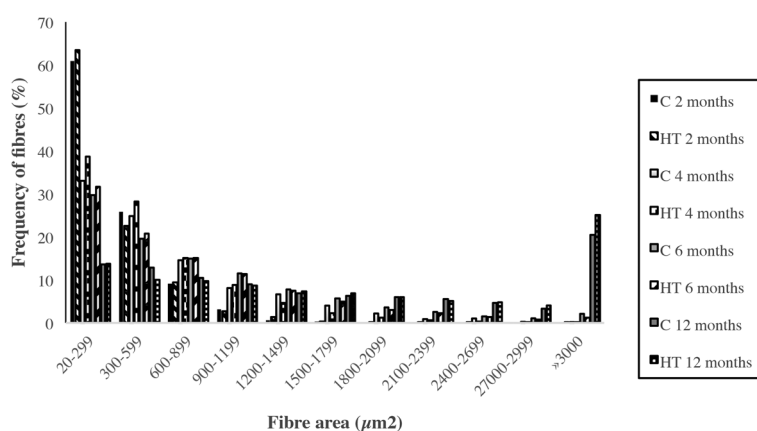


Fig. 1. Frequency of fibre areas in 2, 4, 6 and 12 months old meagre.



## AN OVERVIEW OF DESERT AQUACULTURE IN NORTH AFRICA

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The North African countries (Egypt, Libya, Tunisia, Algeria and Morocco) have a climate with low annual precipitation (from 25 to 200 mm) that meets the criteria to be classified as 'desert'. The low rainfall can at most support a very scanty shrub. The desert covers more than 30% of land area of the five countries. Aquaculture production in the five North African countries has increased 2.2 times in the last ten years (2005-2014), from 0.55 in 2005 to 1.15 million tonnes in 2014. Together the five states contributed 67.4% of the total aquaculture production of the African continent in 2014 (FAO, 2016).

The arid aquaculture facilities in Algeria, Egypt and Tunisia are located inland and use both freshwater and brackish water to produce a variety of ornamental and edible fish such as Nile tilapia (*Oreochromis niloticus*), North African catfish (*Clarias gariepinus*), whiteleg shrimp (*Litopenaeus vannamei*) and micro-algae (mainly *Spirulina* spp.) for biofuels and fat extracts. In the last two decades, the integration of aquaculture with agriculture has become more popular in areas where water is a limited resource. Such integrated systems can produce a source of cheap quality protein and fresh vegetable products with reduced water requirements. Desert aquaculture can provide social benefits, assuring an improved public health and food security for desert communities.

Different examples of desert aquaculture projects using warm geothermal ground water (fresh and brackish) are provided in this paper. In Algeria, water from extensive Nile tilapia culture is used to irrigate small-scale agriculture production; intensive Nile tilapia and North African catfish culture are practiced using Flow-Through and Recirculation Aquaculture Systems, with the addition of whiteleg marine shrimp production using biofloc technology. In Egypt, fresh underground water is exploited for integrated intensive tilapia culture associated with catfish, alpha-alpha (fodder) and sheep production. Intensive culture of European seabass (*Dicentrarchus labrax*) using underground water (26 ppt salinity) is integrated with halophyte plants (*Salicornia* spp., Mediterranean salt brush *Atriplex halimus* and sea blite *Suaeda vermiculata*.) as fodder crops to feed sheep. In Libya, the great potential of desert aquaculture development is considered in association with the Great Man-Made underground network of pipes (2 820 km) and more than 1 300 wells supplying 6.5 million m<sup>3</sup> of freshwater per day in the Libyan Sahara. In Morocco, desert aquaculture started in 2004 and consisted particularly in marine coastal culture of shellfish and exploited by nine shellfish farms which have produced 378 tonnes in 2014. The Pacific oyster (*Crassostrea gigas*) and the grooved carpet shell (*Ruditapes decussatus*) are cultured in the tidal zone of Dakhla bay (Atlantic coast). In addition land-based marine desert aquaculture is under evaluation to assess the potential use of lowlands (called Sebkhass). In Tunisia, the production of Nile tilapia started in the 1970s using the extensive desert aquaculture system in the oasis south of the country and was followed in 2001 by a UNDP integrated agriculture aquaculture project. The production is still low reaching 20 tonnes of Nile and red tilapia in 2015. This document also provides a summary of main issues and constraints affecting the development of aquaculture in the dry inland and marine coasts of North African countries.

## SKIN IRRITATION PROBLEM TO SEAWEED FARMERS IN TANZANIA COASTAL AREA

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Coastal communities depend on fisheries as their main source of income. With the fast rising, coastal population and technological advancement, pressure on the wild marine resources is increasing thus leading to overexploitation. Many people are looking at seaweed farming as a possible livelihood activity that would provide them with an alternative source of income. Skin irritation is among the health problems that has been affecting ocean users such as swimmers, fishers as well as seaweed farmers, due to harmful Cyanobacteria. Exposure of humans to Cyanobacteria in the environment is associated with irritant contact dermatitis, as well as eye and respiratory irritation.

The objective of the study was to identify the factors that cause skin irritation problems, understand the exact season when farmers experience it, and to investigate medication used by farmers when they experience the problem.

Data were collected using a semi- structured questionnaire, in six different villages for three months. Two study sites were in coastal mainland Tanzania and four in Zanzibar Island. The data from the questionnaire survey (N=240) were coded and entered in SPSS, and statistical analyses were carried out. Tabular and graphical analyses of the data were the starting point for data analyses. Frequency tables of ordinal and nominal variables and descriptive tables for scale variables were generated, Chi square test was done to check significant differences in variables.

Skin irritation being a major health problem to seaweed farmers was significant ( $p < 0.05$ ) across the villages. (Table 1). Hot season (83.3%) was identified as the time when skin irritation is high cause of growth of harmful cyanobacteria due to rise in sea surface temperature. It was found that, although some of the famers went to the hospital when they got skin irritation, no specific medication was used to cure the problem, they mostly use local treatments such as application of virgin coconut oil mixed with natural herbal. There is decrease in seaweed production due to some people quit the farming as a results of skin irritation diseases.

TABLE1.Seasons when skin irritation occurs,

| Health Problems                            | Frequency | Percentages (%) |
|--|-----------|-----------------|
| Skin Irritation                            | 135       | 56.3            |
| Eye/Nose Disease                           | 73        | 30.4            |
| Respiratory Disease                        | 14        | 5.8             |
| Paralytic Disease/Death                    | 3         | 1.3             |
| Stomach Disease                            | 8         | 3.3             |
| Other Diseases<br>(Fungus/Injury/Backpain) | 37        | 15              |

## DETERMINING PRODUCTION CHARACTERISTICS OF DUSKY KOB *Argyrosomus japonicus* GROWN IN SEA CAGES UNDER COMMERCIAL CONDITIONS IN RICHARDS BAY, SOUTH AFRICA

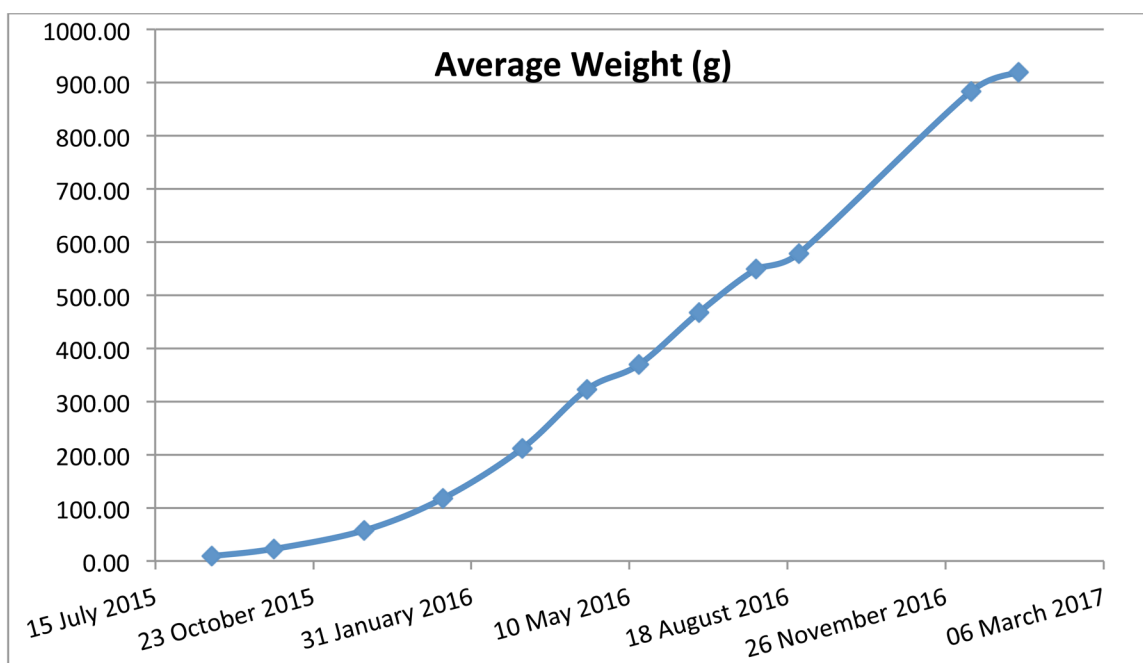
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With aquaculture in South Africa being in its infancy, there are many questions regarding the production characteristics of potential candidate species. The study is based on the collaboration of the Department of Science and Technology, Stellenbosch University and KwaZulu-Natal Aquaculture Development and assessed the technical, environmental and financial feasibility of farming dusky kob (*Argyrosomus japonicus*) in sea cages in Richards Bay, KwaZulu-Natal province. Fish were grown offshore in four surface gravity type cages moored in a 4 x 1 drag embedment anchor based mooring grid. In August 2015, 25 000 dusky kob juveniles of 9 g were stocked in the cages. Fish were fed twice a day with commercial marine finfish extruded diets manufactured by a South African animal feed company.

Significant mortalities were experienced after net changing (2.15%) and fish grading (8.65%) events. It has been reported that this occurrence is a common problem among dusky kob farmers. Fish reached a final average weight of 920 g after 17 months at an average water temperature of 21°C. The average FCR over the entire course of production was 2.09.

This is a preliminary report as this study will also assess the effect of fish size on fillet yield and chemical composition, as well as the differences between farm-raised and wild-caught fish. It is envisaged that this study would be completed in August 2017.



**Figure: Growth curve of dusky kob (*Argyrosomus japonicus*) over a seventeen-month period**

## **ROLE OF KNOWLEDGE EXCHANGE IN FRESHWATER AQUACULTURE DEVELOPMENT AND WATER RESOURCE MANAGEMENT**

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South African freshwater fish farmers (i.e. rainbow trout, tilapia, catfish, crocodiles and ornamentals) operate in a water constrained environment primarily using their source for irrigation and secondly for fish farming. Adoption of innovative technology to improve productivity and water quality is critical to ensure this sector's growth and sustainability. Knowledge exchange among research scientists, fish farmers and extension officers has been the foundation of dissemination. Therefore, it is imperative to collate information on the use of technology modes, assess the application and impact of freshwater aquaculture training manuals, and interrogate innovative technology to improve production through the efficient use of water and reduction of waste.

Primary data for 52 respondents, including fish farmers, researchers and extension officers were collated using a mixed method approach employing a desktop review, semi-structured interviews and focus group discussions. The current technology transfer modes were determined, as well as challenges and opportunities for improved technology transfer.

Results show that 100% of fish farmers use the internet, 53% access learning institutions for marketing, feed, technical, weather and financial information, whilst 13% use farmer to farmer interactions, and only 9% rely on extension officers. Furthermore, more than 80% of fish farmers were not aware of published aquaculture training manuals. Thus the limited growth and expansion of aquaculture in South Africa is partly explained through these findings.

Future initiatives on knowledge exchange could include public private sector collaboration on research, structuring mentoring programmes for fish farmers and students, using social media platforms and implementing learning exchanges locally and abroad.

## THE RELEVANCE OF POSTGRADUATE QUALIFICATIONS TO AQUACULTURE RESEARCH AND DEVELOPMENT IN AFRICA

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Aquaculture is now one of the leading industries in animal protein production. This phenomenon has increased the demand to accelerate aquaculture operations. Developed economies around the globe have been unprecedented in their efforts to explore aquaculture opportunities for sustainable economic growth and development. However, the same success has been slow in emerging economies, with Africa not reaching their aquaculture development goals. For decades it has been postulated that reasons for low growth can be ascribed to *inter alia*, lack of quality seeds and feeds, insufficient institutional support and funding, and inadequate skills. This *status quo* has not changed dramatically in recent times.

Many African aquaculturists have received postgraduate qualifications at distinguished institutions around the world. Therefore, questions posed were: What is the relevance of such specialised higher education and training to the success of aquaculture research and development in Africa? To what extent is knowledge adopted and implemented at ground-level? Up and coming fish farmers are highly dependent on the support and expertise provided by the government, private and community sectors. Thus, qualified personnel in these spheres are directly linked to the success of aquaculture in Africa.

For postgraduate training the success rate for both doctorate and masters is quite high. However, many students did not complete these higher degrees in the minimum required time, i.e. two years for a masters and three years for a doctorate. Many students were also found to be outside an academic environment prior to enrolment and therefore struggled initially with the workload and using information and communication technologies. On completion, skills acquired were varied in nature and across a wide spectrum. The supervisor played an important role in facilitating exposure to skills outside the framework of the research. Furthermore networking between the institution and graduates usually diminished at about two years after graduates have returned home.

It is suggested that a data base of graduates be maintained for referral as well as collaborating on requests for proposals. Collective efforts should be made to table multi-stakeholder projects within five years. Postgraduates should also be encouraged to immediately implement their skills and knowledge via small-scale research projects at their institutions or via a practical knowledge exchange at farm level.

The author will share additional experiences gained in the last 10 years on postgraduate training of Africans. Suggestions will also be made on improving the relevancy of training and mentorship to enhance knowledge exchange and application for aquaculture.

## EVALUATING THE EFFICACY OF THE FALLOWING PERIOD AS A MITIGATION TOOL AT HARD-BOTTOM AQUACULTURE SITES IN NEWFOUNDLAND, CANADA

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Fallowing periods have been shown to be the best management tool for facilitating ecosystem recovery at finfish aquaculture sites located over soft sediments. However, where substrates beneath aquaculture cages are hard and patchy, such as in Newfoundland (NL), Canada, recovery processes and time frames are poorly understood. In NL, the footprint of organic enrichment at the seafloor is determined using video monitoring and the presence of visual indicators: flocculent deposition, barren stations, bacterial mats and opportunistic polychaetes. Here, we characterise changes in benthic communities and in the area covered by visual indicators during different fallowing periods to better understand recovery processes. Video monitoring was performed over three consecutive years at 3 aquaculture sites experiencing different lengths of fallowing.

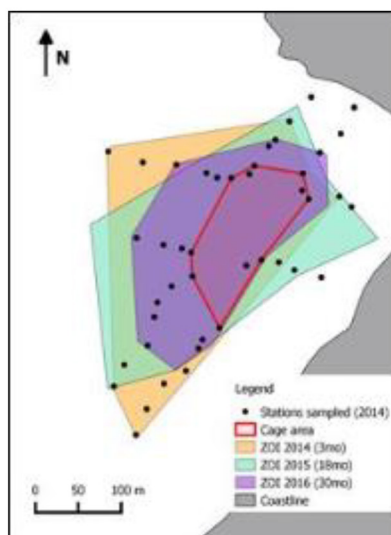
Opportunistic polychaetes and bacterial mats were present at the time of harvest and remained apparent after 30 months of fallowing (Site 1), whereas barren stations and presence of flocculent were observed until 62 and 66 months (Site 2 and 3). Moreover, after 62 and 66 months of fallowing (Sites 2 and 3), the diversity of visible (non-indicator) organisms remained low, suggesting little to no recolonization.

During fallowing, the zone of impact around aquaculture sites decreased slowly with time (Fig. 1). Considering visual indicators separately, the area covered by opportunistic polychaetes decreased more rapidly than bacterial mats. A return to baseline conditions (i.e. with a greater epibenthic diversity) might not take place after 92 months of fallowing, principally due to the considerable change in substrate composition (from hard to soft), but also because of the deep, cold NL environment.

This research helps improve our understanding of the processes of fallowing in NL aquaculture sites and provides advice concerning the duration of fallowing periods that can allow recovery from organic enrichment.

**Figure 1:**

Area covered with visual indicators (zone of impact, ZOI) at Site 1 based on video collected from sampling stations after 3, 15 and 30 months of fallowing. Sampling points extend up to 200 m from cage edge.



## SPERM MOTILITY PARAMETERS OF FAT SNOOK, *Centropomus parallelus*, OF SEMEN CONTAMINATED WITH URINE

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The Fat snook, *Centropomus parallelus*, is a native fish that presents great potential for culture in Brazil, however, technological aspects related to the reproductive processes need to be further studied, such as the processes that influence sperm movement. The objective was to evaluate the influence of urine on the spermatoc parameters of the *C. parallelus*.

Semen from 14 males of *C. parallelus* was collected using an automatic micro-pipette of 1 to 10 µL. After collection the spermatozoa were active with marine water (30ppt) in a ratio of 1:200 (semen: water). A 10x objective of trinocular microscope coupled with a Baster camera (model acA640-120gc), connected to the computer was used to capture the videos. The videos were captured by Basler Pylon Camera software (baslerweb.com) at 100fps (658x492 pixels) in \*.avi format. The edited image sequences (100 files=1s at 20s after activation) were uploaded by IMAGEJ (<http://rsbweb.nih.gov/ij/>) and compiled by the CASA application. The males that presented at the time of collection the mixture of semen and urine were evaluated separately from those that did not present urine.

It was verified that six males (101.3±22.1g, 22.9±1.5cm) presented seminal production (20.8±15.9µL) without contamination with urine. The other eight males (106.0±14.2g, 22.5±1.1cm) presented seminal production (17.9±15.8µL) with contaminated semen. It was observed that the uncontaminated sperm showed a sperm movement of up to 12 minutes (720s), and in 13 minutes all spermatozoa had ceased their movement. As a conclusion, it was verified that urine is an activator of the sperm cells of *C. parallelus* and in procedures of artificial reproduction should avoid to the maximum its contact with the semen.

Table 1. Values (mean ± standard deviation) of sperm parameters of *Centropomus parallelus*, of semen considered contaminated with urine (n=8) and uncontaminated (n=6) with urine.

| Parameters                | Uncontaminated | Contaminated   | <i>t-value</i> | <i>df</i> | <i>p-value</i> |
|---------------------------|----------------|----------------|----------------|-----------|----------------|
| MOT (%)                   | 87.57±8.03     | 15.85±16.99    | 9.50           | 12        | 0.00000        |
| VCL (µm s <sup>-1</sup> ) | 209.25±28.47   | 148.80±53.41   | 2.50           | 12        | 0.02784        |
| VAP (µm s <sup>-1</sup> ) | 147.61±40.55   | 87.53±45.92    | 2.54           | 12        | 0.02582        |
| VSL (µm s <sup>-1</sup> ) | 128.87±37.94   | 73.22±35.30    | 2.83           | 12        | 0.01521        |
| STR (%)                   | 86.85±4.06     | 86.39±11.24    | 0.09           | 12        | 0.92590        |
| WOB (%)                   | 69.48±12.04    | 56.12±16.38    | 1.68           | 12        | 0.11885        |
| PROG (mm)                 | 2107.52±610.89 | 1198.70±609.49 | 2.76           | 12        | 0.01733        |

MOT: motility rate, VCL: Curvilinear velocity, VAP: Average path velocity, VSL: Straight line velocity, STR: straightness, WOB: wobble, PROG: progression, *t-value*: value of calculate t, *df*: degrees of freedom, *p-value*: probability of error according *t test*.



## HEAVY METALS (Cu AND Pb) IN THE FISH TISSUES FROM KOSI A RIVER OF UTTARAKHAND INDIA

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### Abstract

The research work was planned to determine the level of selected heavy metals ( Cu and Pb ) in the fish tissues (gills, liver, kidney and muscles) from the Kosi river of Uttarakhand. Kosi Rivers were examined from December 2015 to April 2016. Two most commonly caught fishes were selected from the river, i.e. *Labeo dyocheilus*, *Channa. Punctatus*. The result of study gives important information on the metal concentration present in fish tissues of the rivers. The concentration of Cu and Pb exceeded the standard permissible limit in Kosi River water.

### Introduction

Fish is widely consumed as a delicious food and a rich source of protein. Fishes were constantly exposed to toxic pollutants in contaminated water and sediment. Fish accumulate heavy metals directly because of the intimate contact they had with the aquatic medium and also because they had to extract oxygen from the medium by passing enormous volumes of water over their gills. Fishes could be used as excellent indicators of heavy metal pollution in aquatic ecosystem. Knowledge of heavy metal concentrations in fish tissues would be important in formulating future management strategies for safe consumption of fish.

### Material and methods

Study Area- Heavy metals were estimated in water, sediments, and fishes of two rivers of Uttarakhand. Kosi River at Kashipur (Distt. U.S. Nagar).

Fish species selected - *Labeo dyocheilus* , *channa punctatus*.

Heavy metals estimated under AAS- Four heavy metals, Lead (Pb) and copper (Cu) were determined in the fish tissue using Atomic Absorbance Spectrophotometer (Thermo Scientific iCE 3000 Series).

### Result and discussion

In the present study, accumulation levels of cadmium, copper, lead and iron were determined in gills, liver, kidney and muscles of two most common edible fishes in local area, *Labeo dyocheilus*, *Channa punctatus*.

The mean concentration of copper found in gills of *L. dyocheilus* was  $1.306 \pm 0.138$  mg/kg, in *C. punctatus*  $1.678 \pm 0.131$  mg/kg. The average concentration of copper reported in liver of *L. dyocheilus* was  $1.436 \pm 0.146$  mg/kg, in *C. punctatus*  $1.822 \pm 0.130$  mg/kg. The average content of copper found in kidney of *L. dyocheilus* was  $1.350 \pm 0.151$  mg/kg, in *C. punctatus*  $1.764 \pm 0.138$  mg/kg. The average concentration of copper found in muscles of *L. dyocheilus* was  $1.286 \pm 0.127$  mg/kg, in *C. punctatus*  $1.640 \pm 0.145$  mg/kg. The investigated concentration of copper in the fish tissues was found to be in the decreasing order liver > kidney > gills > muscles. The distribution of copper in fish species reported in the order of *Channa punctatus* > *Labeo dyocheilus*. The W.H.O. guideline for maximum permissible limit of copper in fish is 3.0 mg/kg.

The average concentration of lead found in gills of *L. dyocheilus* was  $1.216 \pm 0.093$  mg/kg, in *C. punctatus*  $1.626 \pm 0.180$  mg/kg. The average concentration of lead observed in liver of *L. dyocheilus* was  $1.336 \pm 0.097$  mg/kg, in *C. punctatus*  $1.802 \pm 0.155$  mg/kg. The average concentration of lead found in kidney of *L. dyocheilus* was  $1.292 \pm 0.112$  mg/kg, in *C. Punctatus*  $1.712 \pm 0.194$  mg/kg. The mean concentration of lead present in muscles of *L. dyocheilus* was  $1.196 \pm 0.115$  mg/kg, in *C. punctatus*  $1.606 \pm 0.200$  mg/kg. The W.H.O. guideline for maximum permissible limit of lead in Fish is 2.0 mg/kg. The concentration of lead in the tissues of fish were observed in the decreasing order as liver > kidney > gills > muscles. Liver was the most preferred site for accumulation of lead. Comparing among the fishes the order was *Channa punctatus* > *Labeo dyocheilus*. In the present study,

(Continued on next page)

Liver accumulates the highest levels of all the metals. A similar trend has been observed in *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* by **Rauf, (2009)** and in *Labeo rohita*, *Ctenopharyngodon idella* by **Malik et al. (2010)**. Liver is the major detoxification organ and many poisonous materials absorbed from the environment are detoxified in the liver **Schreier et al. (1987)**, **Thompson (1990)**. Kidney had been found to be second in accumulation of metals having lesser metal concentration than liver, and more than gills and muscles. These observations were supported by **Alam et al., (2002)**. During the present study, muscle of all the fish species had been found to accumulate lesser metals as compared to liver, kidney and gills. **Yilmaz, (2009)** stated that organs such as the kidney, liver, gills and gonads, are metabolically active tissue, therefore they accumulate higher levels of heavy metals.

#### Conclusion:

The concentration of Cu and Pb exceeds the standard permissible limit in Kosi water so the water was not safe for drinking purpose but it is safe for irrigation. The water from Sharda River was safe for drinking. Liver was the most preferred tissue for accumulation of heavy metals and muscles were least preferred site for metal accumulation in fish. Among the heavy metal investigated the concentration of Pb was higher the Cu. At present, therefore, the fishes from these rivers are safe for consumption. But the level of Pb and Cu is approaching towards maximum allowable limits in *Channa punctatus*. Long-term consumption may pose significant health hazards.

**Table:1 Average concentration of copper (mg/kg) in various tissues of fish species with SD**

| Fish species from Kosi River |                         |              |              |              |                         |              |              |             |
|------------------------------|-------------------------|--------------|--------------|--------------|-------------------------|--------------|--------------|-------------|
| Fish                         | <i>Labeo dyocheilus</i> |              |              |              | <i>Channa punctatus</i> |              |              |             |
| Tissue                       | Gills                   | Liver        | Kidney       | Muscles      | Gills                   | Liver        | Kidney       | Muscles     |
| Average                      | <b>1.306</b>            | <b>1.436</b> | <b>1.350</b> | <b>1.286</b> | <b>1.678</b>            | <b>1.822</b> | <b>1.764</b> | <b>1.64</b> |
| SD ±                         | 0.138                   | 0.146        | 0.151        | 0.127        | 0.131                   | 0.130        | 0.138        | 0.145       |

**Table: 2Average concentration of lead (mg/kg) in various tissues of fish species with SD±**

| Fish species from Kosi River |                         |              |              |              |                         |              |              |              |
|------------------------------|-------------------------|--------------|--------------|--------------|-------------------------|--------------|--------------|--------------|
| Fish                         | <i>Labeo dyocheilus</i> |              |              |              | <i>Channa punctatus</i> |              |              |              |
| Tissue                       | Gills                   | Liver        | Kidney       | Muscles      | Gills                   | Liver        | Kidney       | Muscles      |
| Average                      | <b>1.216</b>            | <b>1.336</b> | <b>1.292</b> | <b>1.196</b> | <b>1.626</b>            | <b>1.802</b> | <b>1.712</b> | <b>1.606</b> |
| SD ±                         | 0.093                   | 0.097        | 0.112        | 0.115        | 0.180                   | 0.155        | 0.194        | 0.200        |

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## RELEASING OF CASPIAN TROUT (*Salmo trutta caspius*) JUVENILES FOR RESTOCKING PURPOSES IN IRANIAN WATERS OF THE CASPIAN SEA

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*Salmo trutta caspius* is a valuable endemic species in the Caspian Sea. As a result of river pollution, destruction of natural spawning regions, poaching and over-fishing, natural populations of Caspian trout have decreased dramatically. There are two migrating forms of spring and fall run with different appearance characteristics which has the most expensive meat (in the market) among Caspian sea fishes.

The Caspian Brown Trout is considered critically endangered in the southern part of the Caspian Sea according to IUCN criteria. This fish is one of the largest trout's in the world. The heaviest specimen reported was 51 kg. The wild trout, which mature sexually in the brackish water of the Caspian Sea, migrate to the rivers to reproduce in mountain spawning grounds with cold, running fresh water.

After 1949 harvest, according to the fishery statistics yearbook (Iranian Fisheries Organization) and even decreased in some years there is no catch statistics. The Shahid Bahonar propagation and culture of coldwater fishes center (Kalardasht, Mazandaran province) release juveniles of Caspian trout into the some rivers of Iranian coastal waters since 1983. This study focused on status of artificial reproduction and release of this species. According to "five-year development plan" it has been targeted to produce about 600000- 750000 pieces of fingerlings for restocking program during the 2011-2012, but the result exceeded of prediction (about 850000- 860000 pieces of fingerlings). Also in 2011-2012, the average weight of brood females and relative fecundity were 3200 gr and 11.38 eggs/gr respectively.

The sex ratio (Male to female), fertilization rate, survival rate and the Conversion percentage of egg to larva were 1: 2, 98%, 91% and 92% respectively. These fingerlings were released in the upstream and downstream area of the Cheshmekileh and Sardabroud rivers. The catch amount of this species was 2-3 tonnes in the Iranian coastal of the Caspian Sea.

## STUDY OF OSMOREGULATION TREND IN *Rutilus frisii kutum* EXPOSED TO CASPIAN SEA WATER

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The study was done in nutrition and live food in aquaculture research station was located in Bandar Anzali Ghaziyan. Juveniles weighted average 0.5, 1, 2.5 gr were randomly selected in three groups Caspian Sea with a salinity of 11 ppt, water 7 ppt and freshwater (with three replicates per group) were included.

To study the microstructure of gill for each treatment, tissue samples by classical histological methods and stained with hematoxylin - eosin slides were prepared. The frequency and location of the enzyme  $\text{Na}^+$ ,  $\text{K}^+$  - ATPase and chloride cells with immunohistochemical localization was performed. Studies micrometric gill chloride cells by software Image tool (version 2.0) was performed. Data analyzed by one-way ANOVA (Oneway ANOVA) with Tukey's test was performed. Results of gills chloride cells number per square millimeter of the surface gills in *Rutilus frisii kutum* fingerlings showed that number of cells in 0.5, 1 and 2.5 gr juveniles were 9000, 9000 - 10000 and 10070- 12060 cells respectively. Overall, the results of measuring ions and osmotic pressure on the tenth day of treatment, the osmotic potential juveniles 2.5 gr in Caspian sea water and all groups except the juveniles 0.5 gr in water of 7 ppt confirmed.

## GLOBAL EDUCATION EFFORTS TO ENSURE AN AQUATIC VETERINARY WORKFORCE TO ASSIST AQUACULTURE BIOSECURITY

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With increasing impacts of diseases on aquaculture production in all countries, the need for a well-trained aquatic veterinary workforce<sup>1</sup> has become a global imperative. However, numerous educational efforts are underway ensure that sufficient numbers are available to support aquaculture industries and producers, governmental agencies that support or regulate aquaculture, and a myriad of supporting industries that provide therapeutic products, laboratory diagnostic services, and a number services to prevent, control or eradicate diseases. Without this infrastructure, sustainable and economically viable aquaculture will simply not thrive.

In an effort to provide direction to countries on determining the needs, deficiencies, and a pathway to ensure countries have a well-trained veterinary workforce, the World Organization for Animal Health (OIE) has developed a process evaluate and assist building capacity (including education)<sup>2</sup> to ensure a country's veterinary services comply with OIE standards. Similarly, several international organizations (e.g. the World Veterinary Association, the Federation of Veterinarians of Europe, North American Veterinary Medical Educational Consortium) are making strides to ensure veterinary education across the world is standardized, and meets contemporary societal needs. While some address aquatic veterinary education needs, that of the World Aquatic Veterinary Medical Association (WAVMA) stands out. In order to identify the core knowledge, skills and experience (KSEs) needed for those making up an aquatic veterinary workforce, and recognize those with sufficient KSE obtained through a variety of educational opportunities, WAVMA has developed an Aquatic Veterinary Certification (CertAqV) Program. This program specifically identifies 9 core subject-matter areas, that are directly relevant to providing aquatic veterinary services to aquaculture producers. These cover clinical and non-clinical subjects specifically focused on aquatic health issues, including: anatomy and physiology; environmental factors; aquaculture industry structure and function; pathobiology and epidemiology or important diseases; clinical and laboratory diagnostics; the availability and appropriate use of therapeutic and biologic agents; public health, zoonotic diseases and seafood safety; international and national legislation, regulations and standards; and, principles of welfare and humane treatment of aquatic animals.

Preliminary surveys evaluating available educational available, suggest North America, Australia/New Zealand and Western Europe have sufficient opportunities to provide an adequate aquatic veterinary workforce, whereas other regions need improvement. However, with the assistance of the Council on International Veterinary Education, additional work that will continue through 2018, on evaluation aquatic veterinary education in Africa, Asia, Eastern Europe, South America, and the Middle East will help elucidate in all global regions.

A veterinary workforce includes veterinarians and para-veterinarians (veterinary technicians or nurses, fisheries biologists, and other research scientists who provide important and vital laboratory and other supportive services) who collectively provide the services needed to ensure the health and welfare, and implement optimal measures for the prevention, control and eradication of animal diseases. In most countries, these individuals need to be licensed or registered. DeHaven W.R. & A.D. Scarfe (2012). Professional education and aquatic animal health – A focus on aquatic veterinarians and veterinary para-professionals. *Proc. OIE Global Conference on Aquatic Animal Health Programmes. Panama, 28–30 June 2011*. World Organisation for Animal Health (OIE), Paris. pp: 139-154.

The OIE's Performance of Veterinary Services (PVS) Program includes processes and tools for evaluating gaps, and pathways, legislation, statutory bodies, twinning and other programs for improving veterinary services

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(Endnotes)

1 A veterinary workforce includes veterinarians and para-veterinarians (veterinary technicians or nurses, fisheries biologists, and other research scientists who provide important and vital laboratory and other supportive services) who collectively provide the services needed to ensure the health and welfare, and implement optimal measures for the prevention, control and eradication of animal diseases. In most countries, these individuals need to be licensed or registered. DeHaven W.R. & A.D. Scarfe (2012). Professional education and aquatic animal health – A focus on aquatic veterinarians and veterinary para-professionals. *Proc. OIE Global Conference on Aquatic Animal Health Programmes. Panama, 28–30 June 2011*. World Organisation for Animal Health (OIE), Paris. pp: 139-154.

2 The OIE's Performance of Veterinary Services (PVS) Program includes processes and tools for evaluating gaps, and pathways, legislation, statutory bodies, twinning and other programs for improving veterinary services



**IMPLEMENTING PRACTICAL BIOSECURITY THAT MEETS OIE STANDARDS AND REGULATIONS FOR CONTAGIOUS DISEASES PREVENTION, CONTROL & ERADICATION – AN OVERVIEW**

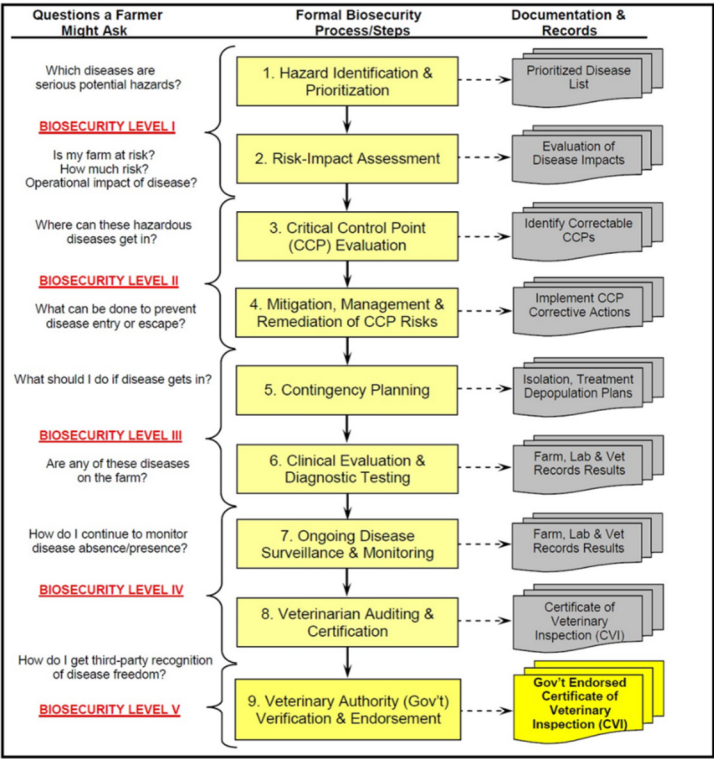
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Facing progressively increasing risks and impacts of disease on aquaculture productions in all countries, over more than a decade at numerous conferences, symposia and workshops, a large number of individuals as part of the International Aquatic Veterinary Biosecurity Consortium (IAVBC) have discussed and debated what procedure that should be incorporated into biosecurity programs. A key feature has been determining which procedures will meet International Standards and National regulations. In balancing these requirements with practical approaches that aquaculture producers can implement, and are effective and useful for all stakeholders around the world (from producers to governmental regulators), the following were recognized as priorities for all biosecurity programs:

- a) be practical and economic;
- b) focus only on infectious and contagious diseases;
- c) include procedures that address disease prevention, control and eradication in definable epidemiological units;
- d) be based on well-established, sound scientific-justifiable veterinary procedures;
- e) incorporate internationally accepted standards in the OIE Code and Manual; and,
- f) involve public-private partnerships and collaboration between producers, aquatic veterinarians and paraveterinary professionals, and governmental regulators.

The IAVBC has tested the procedures in Figure 1 with stakeholders at several conferences and workshops in Norway, South Africa, Chile, and elsewhere, that involve an integrated approach for developing, implementing, auditing and certifying effective aquaculture biosecurity program. At the core of a biosecurity program is defining an epidemiologic unit (EpiUnit; a defined population of animals, separated to some degree from other populations, in which infectious and contagious diseases can be easily transmitted – e.g. a tank/pond, farm, state/province, zone, region or country) that would be appropriate for implementing, auditing and certifying an effective biosecurity program intended to prevent, control and possibly eradicate any infectious disease.



## SYNERGISTIC EFFECT OF TWO BROWN SEaweEDS ON PACIFIC WHITE SHRIMP RESISTANCE TO THERMAL SHOCK AND WHITE SPOT DISEASE

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In previous study, shrimp fed 4% of *Undaria pinnatifida* dry biomass showed an overall improvement of hemato-immunological parameters and higher survival after White Spot Syndrome Virus (WSSV) challenge (48% against 72% of control), while shrimp fed different levels of *Sargassum filipendula* had similar immune response to control. On the other hand, shrimp fed 0.5% and 2% of *S. filipendula* showed higher survival after thermal shock (about 97% against 43% of control), whereas *U. pinnatifida* increased shrimp susceptibility to thermal shock. Thus, this study aimed to evaluate lipid profile of hemocytes from *Litopenaeus vannamei* fed different combinations of brown seaweeds *U. pinnatifida* and *S. filipendula* by MALDI-TOF, in order to evaluate their synergistic effect on thermal shock and WSSV challenge resistance.

Shrimp ( $11.0 \pm 0.2$  g) were reared under two settings, during 15 days for thermal shock trial and 21 days for viral challenge, all in 400 L tanks (50 shrimps/tank), under constant aeration and temperature ( $\pm 29$  °C), 100% of daily water exchange and feeding four times day<sup>-1</sup>. Experimental diets had different combinations levels of both seaweeds: 0.5%/1%, 0.5%/2% and 0.5%:4% of *S. filipendula* (S) and *U. pinnatifida* (U), respectively. Control group were fed the same diets without seaweed addition. At 16<sup>th</sup>, shrimp were submitted to thermal shock, passing from seawater at  $\pm 29$  °C to seawater at 11.5 °C for 1h and then back to  $\pm 29$  °C, and kept for 24h to estimate the mortality. At 22<sup>nd</sup>, shrimp were challenged with WSSV and kept for 96h to estimate the mortality. Hemocyte sampling for MALDI-TOF were performed before and 15 min after thermal shock, and before and 24h after infection. MALDI MS/MS LIFT were performed for lipid identification and <http://www.lipidmaps.org> database were used to confirm the assignment of lipid species.

All treatments had similar mortality after thermal shock, about 70%. Thermal shock caused great impact on hemocyte lipid profile. In general, lipids from m/z 500 to 700 reduced after thermal shock, by contrast lipids higher than m/z 700 increased. MALDI MS/MS identified some lipids under m/z 700 as acyl carnitine (CAR), lysophosphatidylcholine (LPC) and ethanolamine (LPE), phosphatidylcholine (PC) and ethanolamine (PE) with short chain fatty acids, while above m/z 700 mostly PC with long chain fatty acids. Cumulative mortality of 0.5S/2U and 0.5S/4U treatments after WSSV infection were lower (28.1 and 31.3%, respectively) than control (78.1%). Contrary to thermal shock, lipids from m/z 500 to 700 were upregulated, these lipids were identified mostly as being monogalactosyldiacylglycerol (MDGD), CAR, LPC, LPE, PC and PE with short chains fatty acids, while lipids higher than m/z 700 were downregulated, which were mainly comprised by PCs and one LPC, all with long chain fatty acids. Our results indicates that the effects of thermal shock and WSSV challenge on hemocytes were associated to membrane fluidity and lipid metabolism. Additionally, we observed a synergistic effect of both brown seaweeds on shrimp resistance to thermal shock and especially to WSSV infection.

## A COMPARATIVE STUDY OF THE GROWTH PERFORMANCE OF *Oreochromis Mossambicus* AND *Tilapia Rendalli*

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The growth performance of *Tilapia Rendalli* and *Oreochromis mossambicus* was compared over a period of 12 weeks. A total of 10 *Oreochromis mossambicus* and 10 *Tilapia rendalli* fingerlings both with mean body weight of 1.9 g were separately stocked in 375 L glass aquaria feeding on macrophytes. In the second treatment, 10 *O. mossambicus* and 10 *T. rendalli* fingerlings with mean body weight of 1.3 g and 0.9 g respectively were stocked in another 375 L glass aquaria feeding on phytoplankton. A third experiment was set up and contained 10 *Oreochromis mossambicus* and 10 *T. rendalli* both with mean body weight of 0.9 g fed on pellets in fibreglass tanks. During the experiment water quality parameters were monitored on a weekly basis and the mass of the fish was measured at three week intervals. *Tilapia rendalli* feeding on macrophytes obtained a faster growth rate (3.5 g) as compared to *Oreochromis mossambicus* (2.4 g). However, *Oreochromis mossambicus* obtained a faster growth rate (4.2 g) as compared to *Tilapia rendalli* (3.7 g) when feeding on phytoplankton. *Oreochromis mossambicus* feeding on pellets obtained faster growth rate (6.7 g) than *Tilapia rendalli* (6.1 g) and had a better food conversion ratio (1.70) than *Tilapia rendalli* (1.78). This indicates that both species grew significantly faster when supplied with their natural food items. The implication of these results in the context of tilapia culture in South Africa is discussed.



## TILAPIA INDUSTRY IN HAWAI'I: AN UPDATE

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Tilapia are among the most widely produced and commercialized food fish in the world. Originally introduced in Hawaii as a weed control measure, tilapia consumption in the state has recently increased, reaching around 780,000 lbs in 2015. Locally produced tilapia account for 10.2% of the total tilapia consumed in the state, and is primarily destined to the live fish market. Other sources of tilapia include frozen fillet, mainly imported from China, and fresh fish on ice from Taiwan and US mainland.

Challenges to the industry include restrictive regulation on the importation of new species/ strains, high cost of feed, and competition with imports. Recent progress to boost the tilapia industry have included: 1) efforts to facilitate importation of Nile tilapia into Hawaii; 2) the research and development of locally produced feeds based on agricultural byproducts and food wastes; 3) the implementation of system technologies and integration with aquaponics systems; and 4) the development of a commercial feed mill.

This update also provides historical context of tilapia aquaculture and future outlook of the industry in Hawai'i.

## **HORMONAL CONTROL OF GROWTH AND OSMOREGULATION UNDER DYNAMICALLY CHANGING SALINITIES IN MOZAMBIQUE TILAPIA *Oreochromis mossambicus***

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Estuarine fishes, such as the Mozambique tilapia, are suitable models to study the capacity of organisms to acclimate to a range of salinities. Acclimation to salinity is largely mediated by the neuroendocrine system, and involves the regulation of pituitary hormones involved in growth and osmoregulation. Mozambique tilapia reared at the Hawaii Institute of Marine Biology grow faster in seawater (SW) than in fresh water (FW). The elevated growth observed in SW has been shown to be mediated, at least in part, through the growth hormone/insulin-like growth factor (GH/IGF) system. On the other hand, transfer from SW to FW triggers the release of prolactin (PRL), which acts to promote ion uptake and maintain salt-and-water balance.

To date, most studies have reared fish in a steady-state environment, (e.g., FW or SW), or following one-way transfer between salinities, an approach that does not reflect actual conditions of their native habitat, which is characterized by tidal variations in salinity. We compared plasma osmolalities, plasma PRL levels, pituitary PRL mRNA levels, and branchial PRL receptor mRNA levels in tilapia reared in FW, SW, and under a tidal regimen (TR), characterized by changes in salinity between FW and SW every six hours. We also measured the growth rate, feed conversion ratio (FCR), condition factor (K), gene expression of GH in the pituitary, and GH receptor and IGFs in the liver and muscle.

In fish reared in TR, while the expression and plasma levels of GH and PRL were steady, the expression of their receptors in target tissues changed dynamically between each phase of the tidal cycle. Moreover, tilapia reared under TR showed higher growth rates and pituitary GH mRNA expression, and lower FCR and K, when compared with either FW- or SW-acclimated fish. This study indicates that tidal fish are able to compensate for large changes in external osmolality while growing faster and maintaining hormones involved in growth and osmoregulation within a narrow range.

## POTENTIALITY OF DAMS THROUGH CAGE FARMING & ECO-TOURISM INDUSTRY IN SPEDU REGION, BOTSWANA

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The SPEDU Region is endowed with the largest number of man-made water bodies in Botswana as well as natural water causeways in the form of rivers. The country's major dams of Thune, Dikgatlong, Lotsane and Letsibogo and a major secondary dam the Dikabeya constitute over 50% of the country's dams. The Region has identified through SPEDU good potential for a number of small dams (dams with at least 1-million-liter capacity). The small dams are intended to be used as production sites for various agriculture and mixed use enterprises which include cage farming and eco-tourism industry. The existence of the Mmadinare Fish Hatchery near the Letsibogo Dam also adds on to the long list of areas of comparative advantage. These factors provide a prima facie case for aquaculture production in the SPEDU Region.

Aquaculture in the SPEDU Region as is the case in all of Botswana is at an infant stage and characterized by some uncoordinated ways of doing things, a fragmented institutional landscape, and a dearth of technical expertise, scattered activity in addition to underutilization of resources. Currently, the Government of Botswana (GoB) has realized the potential of developing the sector in a manner which ultimately will accentuate its potential and translate it to economic gain. The various initiatives include investment in aquaculture as a vehicle which will assist to eradicate abject rural poverty, commercialization, development of a baseline value chain study on the sector's development and refurbishment of the Mmadinare Fish Hatchery. An Aquaculture Advisor has also been engaged by the Office of the President (OP) with a mandate to propel the development of the sector and position it as a major contributor to GDP.

In an effort to tap into this opportunity, the SPEDU Region requires a concerted way of ensuring that the opportunity is harnessed through proper resource management. A value chain approach will be invaluable to bringing this ideal to fruition. In addition, there is need to enhance product and market diversity by optimization of resources and opportunities. This presentation proposes the development of a SPEDU Region Master Plan. This would place the Region at a vintage position to explore and exploit opportunities and hence accentuate the potential of the Region to become a flagship hub for aquaculture production in Botswana.

This Master Plan identifies six (6) main focal strategic areas which are;

- Development and Implementation of a wide cluster driven aquaculture value chain;
- Enhancement of sustainable environmental management of the sector (legal, health, safety, quality, environment)
- Development of a knowledge based aquaculture industry
- Supporting private sector led growth, promoting entrepreneurship in all stages and components of the value chain
- Identify market access opportunities
- Risk, Mitigation

# DETERMINATION OF LETHAL CONCENTRATION OF UN-IONIZED AMMONIA ON JUVENILE DUSKY KOB *Arygyrosomus japonicus* AND THE EFFECT OF NONLETHAL CONCENTRATIONS ON GROWTH PERFORMANCE

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The study was aimed at (1) determining the lethal concentration (LC50) of unionized ammonia (UIA) and (2) studying the effect of non-lethal UIA on juvenile dusky kob growth performance.

**LC50 trial procedure:** Triplicate groups of six fish each ( $\bar{x}$  = 16g;  $\pm$  5.0 g) were respectively kept in separate tanks (12.5 L fish<sup>-1</sup>) for 48 hours at 22°C (pH-7.8) and exposed to one of the following series of static total ammonia (TAN) concentrations (mg L<sup>-1</sup>) - 0 (control), 6, 8, 10, 12 & 14. Results indicated that the UIA exposed LC50 concentration value for dusky kob juveniles is 0.56 mg L<sup>-1</sup>.

**Nonlethal UIA trial procedure:** Triplicate groups of ten fish each ( $\bar{x}$  = 13.3  $\pm$  1.0 g) were respectively kept in separate tanks (7.5 L fish<sup>-1</sup>) and reared for 45 days at 22°C (pH-7.8) in the following series of sub-lethal UIA concentrations (mg L<sup>-1</sup>) - 0 (control), 0.04, 0.08, 0.12 and 0.16. Feed intake was recorded daily and growth measurements were recorded in 15 day intervals. Results (Table 1 - 4) indicate that the growth rate of juvenile dusky kob is not significantly influenced by UIA levels below 0.17 mg L<sup>-1</sup>. Further investigation is required to test sublethal UIA levels on the growth performance of dusky kob.

Table1: Average body mass (in grams  $\pm$  SE) of dusky kob fingerlings

| Day | Unionized ammonia concentration (mg/l) |                             |                             |                             |                             |
|-----|--|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|     | Control                                | 0.04                        | 0.08                        | 0.12                        | 0.16                        |
| 0   | 13.2 $\pm$ 0.2 <sup>a</sup>            | 13.3 $\pm$ 1.4 <sup>a</sup> | 13.3 $\pm$ 0.7 <sup>a</sup> | 13.3 $\pm$ 0.4 <sup>a</sup> | 13.3 $\pm$ 0.9 <sup>a</sup> |
| 15  | 15.9 $\pm$ 0.5 <sup>a</sup>            | 16.6 $\pm$ 1.3 <sup>b</sup> | 15.3 $\pm$ 0.4 <sup>c</sup> | 14.8 $\pm$ 1.3 <sup>c</sup> | 15.2 $\pm$ 1.3 <sup>c</sup> |
| 30  | 20.0 $\pm$ 3.7 <sup>a</sup>            | 20.6 $\pm$ 2.9 <sup>a</sup> | 20.6 $\pm$ 1.4 <sup>a</sup> | 19.8 $\pm$ 3.4 <sup>a</sup> | 24.8 $\pm$ 4.4 <sup>b</sup> |
| 45  | 27.0 $\pm$ 6.9 <sup>a</sup>            | 26.0 $\pm$ 4.6 <sup>b</sup> | 26.1 $\pm$ 2.3 <sup>b</sup> | 25.6 $\pm$ 5.5 <sup>b</sup> | 30.1 $\pm$ 7.5 <sup>c</sup> |

Same letter means in a row (a, b and c) are not significantly different

Table 2: Average feed intake (in grams  $\pm$  SE) of dusky kob fingerlings

| Day | Unionized ammonia concentration (mg/l) |                             |                             |                             |                             |
|-----|--|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|     | Control                                | 0.04                        | 0.08                        | 0.12                        | 0.16                        |
| 0   | n/a                                    | n/a                         | n/a                         | n/a                         | n/a                         |
| 15  | 1.51 $\pm$ 0.2 <sup>a</sup>            | 1.39 $\pm$ 0.2 <sup>c</sup> | 1.21 $\pm$ 0.1 <sup>b</sup> | 1.26 $\pm$ 0.2 <sup>b</sup> | 1.61 $\pm$ 0.4 <sup>a</sup> |
| 30  | 1.65 $\pm$ 0.4                         | 1.46 $\pm$ 0.2              | 1.20 $\pm$ 0.2              | 1.56 $\pm$ 0.5              | 1.76 $\pm$ 0.9              |
| 45  | 1.67 $\pm$ 0.6 <sup>a</sup>            | 1.67 $\pm$ 0.3 <sup>a</sup> | 1.39 $\pm$ 0.4 <sup>b</sup> | 1.40 $\pm$ 0.8 <sup>b</sup> | 1.95 $\pm$ 0.7 <sup>c</sup> |

Same letter means in a row (a, b and c) are not significantly different

Table3: Average weight gain (in grams  $\pm$  SE) of dusky kob fingerlings

| Day | Unionized ammonia concentration (mg/l) |                             |                             |                             |                             |
|-----|--|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|     | Control                                | 0.04                        | 0.08                        | 0.12                        | 0.16                        |
| 0   | 0                                      | 0                           | 0                           | 0                           | 0                           |
| 15  | 1.77 $\pm$ 0.4 <sup>a</sup>            | 2.28 $\pm$ 0.5 <sup>b</sup> | 1.96 $\pm$ 0.4 <sup>c</sup> | 1.49 $\pm$ 0.6 <sup>d</sup> | 0.88 $\pm$ 0.5 <sup>e</sup> |
| 30  | 5.92 $\pm$ 0.7 <sup>a</sup>            | 4.70 $\pm$ 3.0 <sup>b</sup> | 5.42 $\pm$ 0.9 <sup>a</sup> | 5.41 $\pm$ 2.9 <sup>a</sup> | 7.43 $\pm$ 4.4 <sup>c</sup> |
| 45  | 11.8 $\pm$ 1.4 <sup>c</sup>            | 9.40 $\pm$ 6.1 <sup>a</sup> | 10.8 $\pm$ 1.9 <sup>b</sup> | 10.8 $\pm$ 4.2 <sup>b</sup> | 14.9 $\pm$ 8.8 <sup>d</sup> |

Same letter means in a row (a, b and c) are not significantly different

Table 4: Feed conversion ratio (in grams  $\pm$  SE) of dusky kob fingerlings

| Day | Unionized ammonia concentration (mg/l) |                 |                 |                 |                 |
|-----|--|-----------------|-----------------|-----------------|-----------------|
|     | Control                                | 0.04            | 0.08            | 0.12            | 0.16            |
| 0   | 0                                      | 0               | 0               | 0               | 0               |
| 15  | 0.11 $\pm$ 0.01                        | 0.08 $\pm$ 0.00 | 0.08 $\pm$ 0.01 | 0.09 $\pm$ 0.02 | 0.11 $\pm$ 0.04 |
| 30  | 0.08 $\pm$ 0.02                        | 0.07 $\pm$ 0.01 | 0.06 $\pm$ 0.01 | 0.07 $\pm$ 0.01 | 0.08 $\pm$ 0.04 |
| 45  | 0.06 $\pm$ 0.01                        | 0.06 $\pm$ 0.02 | 0.05 $\pm$ 0.01 | 0.05 $\pm$ 0.01 | 0.06 $\pm$ 0.01 |



## **AQUACULTURE PARKS: IMPROVING HUMAN WELFARE WITH ENVIRONMENTALLY SUSTAINABLE MARKET-ORIENTED AQUACULTURE**

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Fisheries make a crucial contribution to the country's economy and the wellbeing and prosperity of a number of households and today fish constitutes an important source of nutritious food and animal protein for much of the country's population. Small-scale fish farmers have discovered that adopting new technologies is often not enough to increase their productivity unless the fish value chain for their products is enhanced at the same time. Improved value chains lead to increased production and consumption of fish, especially by poor consumers, and increased income for producers, processors and traders.

Fisheries production is one of the priority areas/commodities chosen by the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) under the Country's commodity based approach to developing agriculture. This proposal's general objective is to contribute to a competitive, profitable job-intensive, environmentally-sustainable, gender equitable and climate-resilient agricultural sector in Uganda, in order to reduce poverty and improve food and nutrition security in the context of the changing climate. The specific objective of the project is to support a sustainable, climate-resilient, productive aquaculture value chain in a comprehensive manner.

The purpose of the project will be to improve food and nutrition security, increase income and improve livelihoods, promote inclusive socio-economic development and protect environment and natural resources, focusing on a market oriented aquaculture value chain targeting the East African region, the Middle East and eventually the EU markets, through a private-public partnership. The cage-based aquaculture park that is designed for tilapia production would be located at Mwena, which is a landing site near Kalangala Island on Lake Victoria, and was chosen because it has a number of infrastructure elements that can be used for the aquaculture park. The pond based Aquaculture Park that is designed for catfish and/or tilapia production would be based on the banks of the River Nile where it leaves Lake Kyoga north of Masindi Port, and was chosen because the river provides a vertical change that can be used to flow water through the park.

The project seeks to achieve the following results 1: The policy and regulatory frameworks affecting the operations of the commercial aquaculture industry improved and implemented (including in terms of mitigating climate change/environmental impacts and contributing to climate change adaptation, and sensitizing and responding to women's and men's needs); 2: Production and Productivity of Aquaculture Fish and Fish products Enhanced, giving priority to locally-developed practices and focusing on smallholder and rural livelihoods and formation of producer groups; 3: Post harvest handling and marketing of aquaculture fish and fish products improved; 4: A Project Implementation Unit established.

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## EXPERIMENTAL EVIDENCE OF ROBUST TOLERANCE OF JUVENILE YELLOWTAIL KINGFISH *Seriola lalandi* TOWARDS PROLONGED ELEVATED CO<sub>2</sub> AND LOW PH IN GROWTH AND FOOD CONVERSION

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Yellowtail kingfish (*Seriola lalandi*) is a high value commercial species, currently being farmed in Asia and Australia. A commercial-scale hatchery production has been established in New Zealand, with further plans for the development of the establishment of a land-based recirculating aquaculture system (RAS) on-growing venture. However, removal of excessive dissolved CO<sub>2</sub> in RAS remains an issue. Prolonged elevated levels of CO<sub>2</sub> and the concomitant reduction in pH have been implicated in reductions in growth and health of farmed fish. The current study examined the effect of 8 weeks exposure of juvenile kingfish (initial mean  $\pm$  SD mass =  $0.88 \pm 0.10$  kg) to dissolved CO<sub>2</sub> levels of 2, 8, 15, 20 mg/L, corresponding to pH 7.9, 7.4, 7.2 and 7.0, representing the range from low to the limits currently prescribed in a range of countries. We recorded survival, growth and food conversion ratio (FCR). Fish were fed at approximately 2.5% body mass per day. Growth including 18 days prior to pH treatment to the end of the trial showed that the fish more than doubled their body mass in 78 days from an average of 1.26 kg to 2.63 kg, with no significant differences between treatments. We also found no significant differences in specific growth rate (mean  $\pm$  SE =  $9.54 \pm 0.10$  g/kg.d) or biological feed conversion ratio (mean  $\pm$  SE =  $1.63 \pm 0.02$ ), normalised to an estimated average weight per tank. Analyses of haematological health and stress parameters are pending. Nevertheless, the available data show that juvenile yellowtail kingfish have environmental tolerances that make them particularly suited for RAS.

## EFFECTS OF DIETARY LIPID, L-CARNITINE AND L-LYSINE SUPPLEMENTATION ON THE GROWTH PERFORMANCE AND BODY COMPOSITION OF *Epinephelus lanceolatus* LARVAE

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The effects of dietary lipid, L-carnitine and L-lysine levels on the growth performance and fatty acid profile of muscle and liver of 0.08 g grouper *Epinephelus lanceolatus* larvae were investigated. Experimental diets included two levels of (5 and 14%) oil mixture (3:1 fish oil and soybean oil) supplemented with L-carnitine (0 and 0.5%) and with/without 2.83% L-lysine were formulated. Each diet was randomly assigned to three replicate groups of grouper larvae cultured for 42 days. The grouper larvae fed diets containing 14% lipid had significantly higher weight gain than those fed diets containing 5% lipid. The grouper larvae fed diets supplemented with L-carnitine had significantly higher weight gain than those fed diets without L-carnitine. Dietary L-lysine supplementation did not affect the weight gain of grouper larvae. The grouper larvae fed diet containing 5% lipid supplemented with L-lysine and L-carnitine had the worst weight gain among all treatment groups. The fatty acid compositions of muscle and liver of grouper larval were influenced by dietary lipid, L-carnitine and L-lysine. The n-3 highly unsaturated fatty acid (n-3 HUFA) of muscle and liver of grouper larvae fed diets supplemented with L-carnitine or L-lysine was significantly lower than that of grouper larvae fed diets without L-carnitine or L-lysine. The hepatosomatic index (HSI) of grouper larvae decreased with increasing dietary lipid and L-carnitine. The HSI did not be affected by L-lysine supplementation. Lipid levels of muscle and liver of grouper larvae increased with increasing dietary lipid. However, lipid levels of muscle and liver of grouper larvae fed diets supplemented with L-carnitine or L-lysine were significantly lower than those of fish fed diets without L-carnitine or L-lysine. The protein level of muscle of grouper larvae significantly increased with increasing dietary lipid, L-carnitine or L-lysine. The survival of grouper larvae fed treatment diets ranged from 87 to 100%. This study indicated that diets included high dietary lipid and supplemented with L-carnitine promoted a good growth performance of grouper larvae.

## EFFECT OF DIFFERENT DIETARY PROTEIN SOURCES ON GROWTH PERFORMANCE OF RUFIJI TILAPIA *Oreochromis urolepis urolepis*

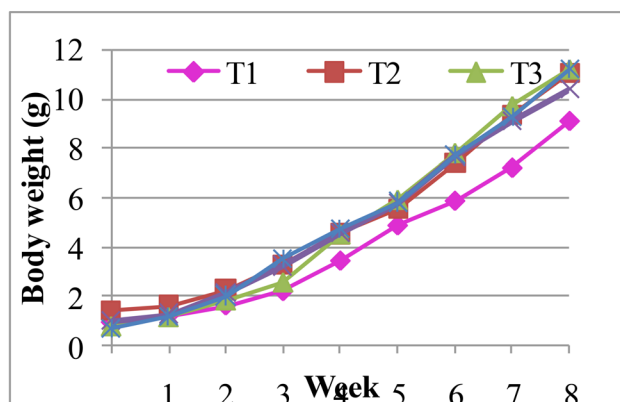
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One of the challenges facing intensive tilapia aquaculture development in developing countries is finding suitable alternatives to fishmeal (FM) from easily available and cheap local dietary protein sources. A 56 days experiment was therefore conducted in 80-liter plastic containers to investigate the suitability of blood meal (BM), sunflower meal (SFM) and Azola meal (AM) as alternative dietary protein sources on growth performance and feed utilization of the Rufiji tilapia, *Oreochromis urolepis urolepis* Norman (1922). Dry pelleted BM, SFM and AM containing 35% crude protein and 10% lipid were used as a protein sources and FM was used as a control in feeding two sets each containing 10 fingerlings with an average weight of  $0.92 \pm 0.0$ . They were fed twice a day at a daily ration of 5% of the body weight. Water parameters monitored during the study were pH, temperature, salinity and dissolved oxygen concentration. No significant differences ( $P > 0.05$ ) were found in growth performance and feed utilization in terms of weight gain, protein efficiency ratio, feed conversion ratio, feed intake and specific growth rate in all the three diets used.

From the results of the present study fish meal can be replaced up to 15%, 45% and 60% with Azolla, sunflower and blood meals respectively without affecting the general growth performance of the experimental fish. Cost analysis showed that fish feeding on BM was most cost effective followed by AM then SFM

**Figure 1: Change in body weight of *O. urolepis urolepis* fed diets containing graded levels of blood meal**



## PROFITABILITY IN AQUACULTURE WITH EMPHASIS ON SHRIMP FARMING

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Aquaculture is an investment option. However, as with all solid investments, this option should only be transformed into action if, through comprehensive market and business planning, it proves to be profitable. Profitable aquaculture enterprises, aqua-businesses, can assume any scale of operations, from micro to industrial. But, at any size, it must be market-driven. For smaller operations, this implies the need for firms to band together to achieve an economic critical mass; forming clusters, pulling down services and attracting better markets. A decision to create a commercial enterprise requires significant research, a business plan and a risk analysis.

Market analysis is essential before setting up of any businesses. It is important analyze potential markets to determine if there is sufficient potential buyers for the chosen product. Area market potential can then be estimated by extrapolating information on the number of buyers within your area.

Adjusting to the market needs by making changes in the production schedule after market analysis will lead to higher profits. Research on a substitute product to be sold in case there is lack of information for the former product.

After estimation of profits is done, the cost to be incurred has to be calculated in advance too. This is because, during the culture period, there will be a cash gap as there will not be any income. By creating a list of expenses to be incurred, this will allow you to budget for it and result in better handling of money flow.

In order to have a sustainable business, it is important to keep track of all the marketing history, opportunity and issues faced and current market situation to plan for the future costs, competitions and problems.

Future goals of the business in the areas of production, profit, growth, and market share have to be listed down to motivate the company to continue to grow.

Despite its romantic allure for some, shrimp farming is a business like any others. Income must be greater than costs to show a profit.

## **REVOLUTION IN FISH FEED AND NUTRITION IN KENYA'S AGRICULTURE INDUSTRY**

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In many agriculture systems the feed delivered to the fish provides all nutritional requirements. Feed is second parameter to water quality and single input with highest cost. Kenya has the potential to produce fish for both domestic consumption and commercial purpose. This potential is partially limited by poor quality fish feed of low FCR. Last three years has seen a revolution in fish feed quality and nutrition. Big manufacturers and researchers have made entrant into the Kenyan market; skretting. Aller Aqua, fish food among others. However, the cost of these feeds remains a major challenge for most small holder farmers. Trials by International Centre for Insect Physiology and Entomology (ICIPE) researchers to introduce cheaper feed by raising colonies of crickets and black soldier fly maggots are on going. Concerns are weather such a feed will be acceptable, by consumers, bearing in mind maggots are not eaten locally. Feed supplement through use of probiotics and periphytons are also being introduced, collaboration of farmers, researches and extension officers will ensure the success of these new technologies



## PRELIMINARY SOCIO-ECONOMIC FINDINGS FROM ASSESSMENT OF THE EPIZOOTIC ULCERATIVE SYNDROME (EUS) DISEASE IN FINFISH IN ZIMBABWE

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This abstract presents preliminary results of an ongoing study in Zimbabwe focusing on assessing Socio- economic impact of the Epizootic Ulcerative Syndrome (EUS) disease on human livelihoods, fish production and productivity, biodiversity and national natural resources. A detailed socio- economic study questionnaire was developed for data collection, and the instrument is currently being administered in the study area of Kavango Zambezi (KAZA) and Great Limpopo (GL) Transfrontier Conservation Areas (TFCAs) of Zimbabwe, through Focus Group Discussions (FGDs), and face to face Key Informant Interviews (KII) of target community members and key stakeholders. In addition, the research teams collected data through making direct observations, when the situation allowed for it, with minimal intrusion. Starting from May 2016 up till January 2017 a reliable preliminary size of responses from the study survey had been received and analyzed.

The results give a general perspective of socio- economic effects of the disease on the targeted rural fishing communities observed over a period of one season and the detailed socio- economic findings and trends reveal that exact economic output requires a continued study over time, although certain fish species have declined. Estimates of direct economic losses were difficult to establish as records of fish catches and sales of fish products were not always available for analysis in Zimbabwe. In Bangladesh for instance, reduced aquaculture and fisheries productivity was demonstrated during times of serious EUS outbreaks, although that study could not positively determine with precision that EUS was the factor that directly caused the decline. A total of 3,658 fish were sampled during the winter sampling season of 2016 in wild capture fisheries, of which 763 fish (21% prevalence) were diseased with EUS typical haemorrhagic lesions. It is however important to note that a prevalence of over 90% was recorded in two distinct water bodies elsewhere in the country during the same sampling period, one within the Kavango- Zambezi (KAZA) drainage area and another outside of the project study catchment area. Seven (7) aquaculture farms culturing mainly *Oreochromis niloticus* (Nile Tilapia) were also surveyed at the same time as part of this study, and a total of 1,120 samples collected with zero disease prevalence recorded.



“This work was conducted within the framework of the Research Platform -Production and Conservation in Partnership (RP-PCP) ([www.rp-pcp.org](http://www.rp-pcp.org))”, and this document was produced with the financial assistance of the European Union, with the facilitation of CIRAD- Dream Project. The contents of this document are the sole responsibility of the authors indicated above and can under no circumstances be regarded as reflecting the position of the European Union.

## THE REVIEW OF AN AQUACULTURE AND FISHERIES PHD BY COURSEWORK CURRICULUM TO SERVE THE NEEDS OF SUB-SAHARAN AFRICA

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Hunger and poverty are major problems faced by the people and governments in Sub-Saharan Africa. This is exacerbated by a lack of critical human resources to spur innovation and exploitation of natural resources and opportunities to enhance livelihoods. The development of fisheries and aquaculture resources has been identified to be key in the fight against poverty. However, there is a lack of skills in these areas with a recent study suggesting that less than 16% of the Sub-Saharan African fisheries scientists at university and government institutions have a PhD-degree and less than 50% hold an MSc degree. Although there are limited opportunities for PhD training in fisheries and aquaculture in Sub-Saharan Africa, the Lilongwe University of Agriculture and Natural Resources (LUANAR) initiated a dedicated coursework PhD program in 2007.

This presentation will report on the progress of a review of this PhD program curriculum, which was funded by the African Caribbean Partnership of the European Union Co-operation Program in Higher Education (Edulink II – Project contract number DCI-AFA/2013/320-302). This review comprised an evaluation by fisheries and an aquaculture experts and was followed by a four-day workshop in Grahamstown in March 2017.

The primary finding of the review was the need to shift from the traditional teacher-centred to a learner-centred approach. This entails the development of clear, fit-for-purpose, learning outcomes (LO) for the curriculum and the development of appropriate (formative and summative) assessments that measure and improve teaching and learning. Several new core courses (e.g., philosophy of science, scientific writing and research integrity and ethics) that aimed to support the preparation of student theses were also recommended.

The next phases of the project require a coordinated effort to review the content of specific courses against the backdrop of the new approach to ensure that each module contributes to fulfilling the learning outcomes, adoption of the revision by LUANAR, and the implementation of the new curriculum.

## THE COMPLEX ROLE OF SMALL SCALE AQUACULTURE TO FOOD SECURITY: THE CASE OF MALAWI

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The meteoric rise and evolution of aquaculture over the past few decades has led to both optimism and apprehension regarding the sector's sustainability and role to food security. Comprised predominantly of small-scale operators, the sector is recognized to play a critical role in supporting livelihoods, contributing to food security and alleviating poverty. However, the ability to achieve these potential benefits is not a given and the transformation of communities adopting aquaculture can be positive, neutral or negative. Assessing the sectors contribution in a systematic way has been an uphill challenge due to the typical part-time and dynamic temporal engagement of operators as well as the complex socio-ecological factors that mediate aquaculture development outcomes. The aim of this study was to explore these gaps by implementing a mixed methods approach guided by the Sustainable Livelihoods framework to advance our understanding of the role of small scale aquaculture to food security. Our contribution to the debate focuses on Malawi, a country where the potential for aquaculture development is reported considerable. This study is based on: 1) key informant interviews; and 2) household questionnaires and the participatory Photovoice method in two fish farming communities; collected during 2014-2015. Overall, findings show that small scale aquaculture contributes positively to local livelihoods through complex pathways to improved food security, improved well-being and reduced vulnerability. However, the type of aquaculture development, gender relations and cultural norms shape development outcomes. Significant social, environmental and economic constraints are also identified that negatively affect the sustainability of aquaculture. Findings further highlight the value of Photovoice in illuminating the role of men and women fish farmers and in capturing their perspectives concerning the impact and sustainability of aquaculture. Our findings have important policy implications and make novel contributions to the on-going debate concerning aquaculture's future and its role to food security.

## OPTIMIZATION OF NUTRIENT INPUT TO INTEGRATED AQUAPONICS SYSTEMS THROUGH MINERAL SUPPLEMENTATION BY WAY OF FISH FEED ADDITIVES

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Aquaponics is an integrated production system with the main goal of sustainable food production in the form of fish and vegetable. It realises a need for sustainable and reliable food production that uses resources efficiently. This system represents a solution to conventional management of water quality in recirculating aquaculture systems by way of wastewater uptake by plants. The main challenge in aquaponics is the imbalance of nutrients between fish and plants grown in the system as fish and plants have different nutritional requirements. Requirements of fish are met through fish feed and those of plants are met by supplementing nutrients, especially trace elements, through nutrient solutions, adding extra costs to the production system. Therefore, there is a need to design a feed that will fulfil a dual role: provide optimal nutrition to the fish and the plants once it has been excreted by fish. The addition of minerals to fish feed could reduce or even eliminate the need to supplement plants with the nutrients in the form of nutrient solutions. Improvement of plant growth through dietary feed additives of fish in aquaponics systems has never been details before.

For this study, supplementation of minerals to fish feed will be achieved by way of feeding trails using the African catfish, *Clarias gariepinus*. The first feeding trial will investigate fish production, fish health and water quality in a recirculating aquaculture system. The minerals, potassium and iron will be supplemented by addition of different fish feed additives from different sources and at different inclusion levels. Potassium will be from potassium diformate (KDF) or potassium chloride (KCl) and iron from amino acid chelated iron (Iron methionine) or iron sulphate ( $\text{FeSO}_4$ ). The second trial will investigate the effect of the best dietary feed additive and inclusion level on a recirculating aquaponics system using catfish and lettuce. Fish production and health, water quality, lettuce growth and quality will be investigated. Results of this study are expected to help improve fish growth while optimising plant production in aquaponics systems.

## RESULTS FROM INTEGRATED RICE-FISH FARMING PILOTS: A POTENTIAL INTEGRATED FARMING SYSTEM IN SIERRA LEONE

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Considering the extent of inland valley swamps (IVS) availability (54,650 ha) in Tonkolili District in Northern Sierra Leone and rice as the primary cultivated crop, the potential to increase fish productivity through integrated rice-fish farming, and therefore increase incomes and food and nutrition security of rural people, is significant. Since there are no documented data on attempts to test integrated rice-fish farming, nine integrated rice-fish pilots in rice fields with extents ranging from 412 to 1,560 m<sup>2</sup> in IVS in five chiefdoms of Tonkolili District were conducted. Two high yielding rice varieties (Nerica L-19 and Rok-34) were planted in equal plot sizes in each rice-fish pilot and trenches to accommodate fish were constructed in the rice fields. Nile tilapia, *Oreochromis niloticus*, of 5-6 g size were stocked at 2 fish/m<sup>2</sup> and fed with an on-farm made feed mixing husk free rice bran (30%), fish meal (fish waste powder) (60%) and cassava powder (10%) twice daily. The pilots were carried out through direct community participation, support from NGO partners, local government field officers, and WorldFish staff using a farmer group structure – composed of a group of research farmers who implemented the pilot, learner farmers who actively observed and learned from the pilot, and a small advisory group – benefiting 1,029 households, including 38% women. Participatory action research processes were set up in each pilot site with impact pathway and theory of change as the base for learning. The reported fish yields ranged between 356 to 1,616 kg/ha, recording a -11% to 304% change from the baseline yield of 400 kg/ha. The rice yields were 984.3 to 7,154.5 kg/ha, recording a -23.1% to 459% change from the baseline yield of 1,280 kg/ha. Eight out of nine pilots (89%) recorded higher rice and fish yields above baseline yields. Positive benefits to costs ratios were found for the rice component (1.06 to 1.70) in 78% of the pilots and for the fish component (1.15 to 1.4) in 89% of the pilots. Eight out of nine of the pilots showed positive benefits to costs ratios (1.15 to 1.70) for both integrated rice and fish commodities, indicating a positive influence of fish on the rice-fish integrated systems with negative benefits to costs ratios of the rice component. In light of the prominent rice agro-ecology and vast areas of IVS in Tonkolili District and the suitable areas for fish farming, the pilot determined that the potential to develop rice-fish farming and aquaculture in the district is substantial. Additional lessons learned from the pilots are discussed in the paper.

## VENTRAL VIDEOGRAPHIC ASSESSMENT OF THE FEEDING BEHAVIOR OF JUVENILE GREENLIP ABALONE *Haliotis laevis* IN RESPONSE TO DIET AND AN ACUTE WATER TEMPERATURE CHALLENGE

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Recently, there has been interest in including dried macroalgae, specifically *Gracilaria* sp. in greenlip abalone diets, dried enriched *Gracilaria cliftonii* inclusion previously led to increased feed intake and weight gain in greenlip abalone. In addition, greenlip abalone have been observed to start eating earlier, in the daylight, when fed diets containing dried *G. cliftonii*. In Australia, greenlip abalone are exposed to water fluctuation, water temperatures nearing the thermal maxima of the species can cause abalone to decrease their feed intake, which can manifest/ exacerbate into nutritional and immunological issues. Targeting diets that increase feed intake, or maintain feed intake during environmental stress events, would be fortuitous for the industry.

This multifactorial study used a ventral videography technique to investigate the feeding behaviour of juvenile greenlip abalone fed either a 0% basal diet or diet containing 10% dried enriched *G. cliftonii* diet. In addition, abalone were exposed to an acute temperature challenge. Abalone were held at 22°C for two weeks, thereafter, during filming the water temperature was raised 1°C day<sup>-1</sup> until a water temperature of 26°C, nearing the animals thermal maxima of 27.5°C, was achieved. Abalone were fed to excess and feeding behaviour was measured under a photoperiod regime of 12 h dark (red light) and 12 h white light. Feeding behaviour was scored in terms of the following activities: quiescence, alertness, moving, feeding, distance travelled, velocity and homing. In addition feed intake was also measured.

There was a significant effect of diet type on the feeding behaviour of abalone. Abalone fed the 10% *G. cliftonii* diet ate significantly more than when fed the 0% basal diet. In addition, there was no pronounced effect of water temperature on the feed intake of abalone fed the 10% *G. cliftonii*. In contrast, when fed the 0% basal diet, feed intake started to decrease as abalone were exposed to water temperatures > 24°C.

This suggests that the use of chemosensory attractants may be beneficial in abalone aquaculture to promote feeding activity. This in turn can translate to improved weight gain, and notably in regards to this study maintain the feed intake of abalone in response to acute environmental changes. This new information may assist in refining feed formulation and feeding practices for the cultured abalone.

## FORMULATING CATFISH FEEDS FOR THE AFRICAN CONTINENT

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The versatility and robustness of African catfish (*Clarias Gaerlepinus*) allow this species to thrive in challenging environments. Although gaining popularity, some constraints limit a production increase. Among them are feed quality and availability.

### Nutritional feed quality

Raw material selection and quality are paramount factors for producing highly digestible and palatable feed. Imbalances in nutrient composition affect the water quality of the pond or tank. For example, feeds giving high feed conversion ratios will result in high amount of faeces. The faeces of African catfish are unstable and difficult to separate from the water. Given relatively low water exchange and high fish biomass per cubic meter, faeces disintegrate into many small particles and pollute the whole water body. Although African catfish can gulp air from the surface, a filled digestive tract and high biomass together with inappropriate environmental conditions can cause high and sudden mortalities on the farm.

### Physical feed quality

While in more sophisticated markets, laboratory feed analyses are common, farmers in many African countries consider physical quality an important measurement. It is therefore required to guarantee stable physical quality – and deliver cost optimized feeds of same size, same shape, same surface and same colour all the time to be accepted by the farmer.

### Availability

In many African countries, fish farmers are not used to constant availability of good feeds. Then products disappear from the market for various reasons. This spoils the farmers' trust in the product and the supplying company. Once feed is unavailable, farmers may quickly turn to another supplier they find more comfort in. Information spreads fast in African markets and within short time, products can become highly requested. At this stage, feed availability becomes the important factor to benefit from positive word-of mouth.

### Summary

Due to the fast-growth of catfish, it can generally quickly be demonstrated, that high-performance feed of stable quality enables faster fish growth and gives fish ready for the market earlier than commonly observed. The feeds now gaining popularity in African markets could also be used for indoor RAS farming. Such a quality level would not be everyone's first thought when considering the ideal product for catfish. However, given the popularity of this fish and its local price, farmers are adapting to better feed to keep this a constant in an otherwise often challenging environment.





## THE EFFECT OF AVAILABILITY OF SHELTER ON SURVIVAL OF THE FRESHWATER CRAYFISH *Cherax cainii* (MARRON) IN TANK CULTURE

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*Cherax cainii* is a high-value, niche product, and ranked second highest of all farmed crustaceans in terms of per kilogram value in 2012 (US\$ 37.60/kg, FAO FishStatJ 2012). Currently farming takes place in pond culture and the industry is still considered developmental. Tank culture for *C. cainii* is of interest as it potentially has advantages over pond culture, *inter alia*, better control of system variables, mitigation of the spread of the species outside the system, and better control and monitoring of production. In tank culture, intensification is usually required but *C. cainii*'s agonistic behaviour may be a hindrance. Crayfish use fighting to establish dominance for better access to shelter, food, and mating opportunities.

Structural complexity (shelter) has been shown to improve survival in other crayfish species by reducing the number of aggressive interactions between conspecifics (but not the intensity of interactions). The provision of shelter is practiced in pond culture and various *C. cainii* production guidelines and tank and pond studies report the use of shelter, however shelter has only been the focus of a few studies. The study aims to investigate the effect of shelter on growth and survival.

*Cherax cainii* production (survival and growth) with and without shelter is being tested over 105 days. Crayfish are fed experimental pellets twice weekly, in excess. Treatments included two size-classes (0+ yr-old (3.5 – 44 g) and 1+ yr-old (50 – 140 g)) and two densities (n = 12 and 37 for 0+ yr-olds and n= 5 and 15 for 1+yr olds), which were tested with and without shelter. Four replicates of each treatment were tested.

Crayfish with and without shelter were recorded using a RaspberryPi development board with NOIR camera module in an underwater housing, equipped with additional infrared lighting for underwater viewing at night. The camera was programmed to record 20 minutes of footage every hour.

Survival rate and specific growth rate will be calculated and differences between treatments will be determined using ANOVA. The number of aggressive interactions in the camera footage will be counted and compared between treatments with and without shelter using ANOVA.

It is hypothesised that shelter will reduce the number of aggressive interactions, thereby reducing mortality rates. The experiment ends ending mid-March 2017.

## **NATIONAL STRATEGIC ENVIRONMENTAL ASSESSMENT FOR AQUACULTURE DEVELOPMENT IN SOUTH AFRICA – GIS ANALYSIS FOR IDENTIFYING OPTIMAL AREAS FOR MARINE AND FRESHWATER AQUACULTURE DEVELOPMENT**

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Geographical information systems (GIS) have increasingly become a crucial tool for planning and managing natural resources, and have been implemented around the world for identifying suitable, sustainable and optimal areas for aquaculture development using a range of environmental, economic, and social parameters.

Some of the key challenges currently facing the still developing aquaculture industry in South Africa include amongst others a high-energy coastline with a limited number of naturally protected sites, limited access to sufficient land space and adequate freshwater resources, high water quality and temperature variations, user conflicts and an overregulated sector.

A national-scale Strategic Environmental Assessment (SEA) was commissioned by the South African government in 2016 to create an enabling environment where sustainable aquaculture development can be promoted contributing to food security and job creation.

As part of the Aquaculture SEA, optimal areas for aquaculture development in South Africa were identified in the marine and freshwater environments using GIS spatial analysis. Parameters considered included existing conditions, uses, and users of the environment, as well as the requirements of aquaculture facilities employing specific operational systems for breeding different species. Challenges included obtaining the necessary detailed information on existing aquaculture facilities and environmental parameters. Environmental data layers were often only available at different scales thus hampering analyses and interpretation.

Identification of optimal aquaculture areas, at a strategic level, aims to i) facilitate the development of aquaculture in an environmentally responsible manner; ii) assist potential aquaculture developers by acting as a high-level development siting tool; iii) maximise the sustainability of new aquaculture development; and iv) to stimulate the industry by reducing regulatory complexity and incentivising development within the identified optimal aquaculture areas.

## ASSESSMENT OF FISH DISEASES AT AQUACULTURE RESEARCH STATION IN PORTUGAL

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Fish diseases are one of the main problems in aquaculture, especially in intensive fish farming where they represent severe annual costs to producers. Proactive measures are important to prevent fish diseases outbreaks. As a preventive approach, we implemented schedule samplings to all the production areas of the Aquaculture Research Station (EPPO). This allow us to anticipate the detection of potential diseases, avoid treatments and prevent fish mortality. EPPO holds a marine fish hatchery, several fiber-glass tanks and earthen ponds and therefore different approaches must be used to prevent fish diseases on these different areas of rearing. The scheduling and type of sampling take in to account the production area and fish size (hatchery: marine fish broodstock and larvae, earthen ponds: juveniles and adults), the water temperature and the critical periods when certain fish diseases occur that can affect the fish species produced. The occurrence of fish diseases at EPPO are distributed according the Figure1. In earthen ponds, used for on-growing, sampling purpose is to assess fish ectoparasitological load, and the periodicity and intensity of sampling is higher in the summer when the temperatures and the probability of ectoparasites occurrence are high. But at larval stages, the priority is to check the ultraviolet system efficiency, and confirm that water is free from bacterial contamination before eggs incubation and larval rearing. The periodicity of sampling are covers whole larval phase (depletion of yolk sac, changes from live to inert food, etc.).

When a fish disease outbreak occurs, a pathological analysis is performed on fish sample, which includes observation of ectoparasites and endoparasites, microbiology analyses of liver, spleen and anterior kidney, histological analysis of these organs that will allow us to detect and identify possible pathogenic agents. If the fish disease was caused by a pathogenic bacteria, antibiogram tests will be performed, and the bacteria will be identified by traditional biochemistry tests and/or molecular techniques.

These approaches allowed us not only to decrease fish loss caused by diseases at EPPO, but also to improve fish welfare at the facilities and provide information to the aquaculture industry.

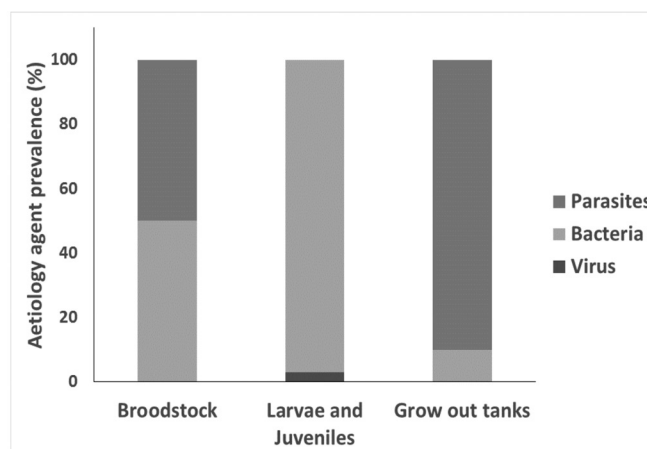


Figure 1 - Prevalence of diseases caused by virus, bacteria and parasites in the different sections of EPPO-IPMA facilities.

## DIETARY SUPPLEMENTATION WITH ISOQUINOLINE ALKALOIDS FOR THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei* WHEN CHALLENGED WITH *Vibrio parahaemolyticus*

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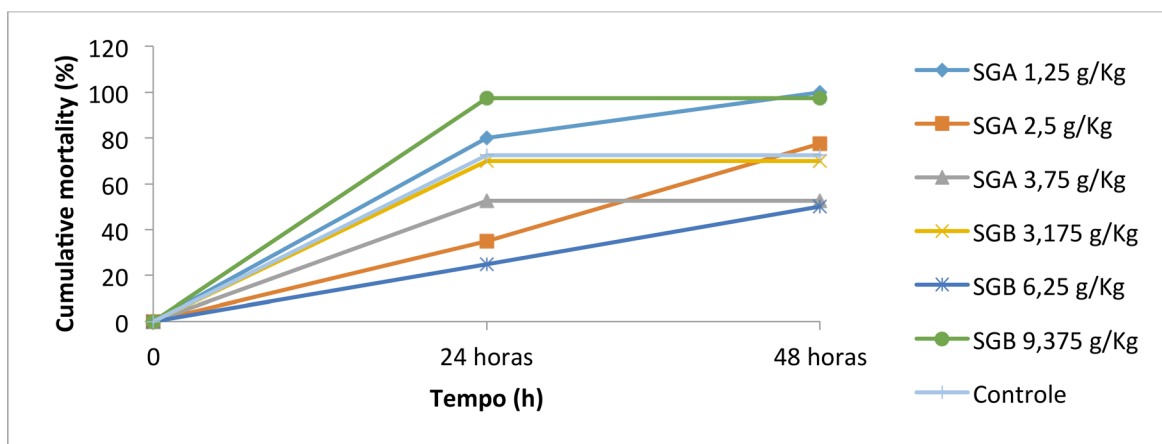
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*Litopenaeus vannamei* is the most farmed shrimp in the world, but one of the main factors that affect shrimp industry is diseases, especially those caused by virus and bacteria. Thus, research using natural compounds extracted from plants (phytotherapeutics/phytogenics) applied as growth promoters and health supplements has been conducted. The objective of this study was to evaluate the survival of Pacific White shrimp fed diets supplemented with different inclusion levels of isoquinoline alkaloids in different formulations (SGA vs. SGB) in shrimp experimentally challenged with *Vibrio parahaemolyticus*.

Experimental diets with different levels of dietary supplementation were used: SGA (1.25, 2.5, 3.75 g / kg), SGB (3.125, 6.25, 9.375 g / kg) and a control (diet without supplementation). During six weeks, shrimp were fed diets and then 40 shrimp from each treatment (280 total) were used for the experimental challenge. Animals were injected with 100µL of *V. parahaemolyticus* ( $9 \times 10^7$  CFU mL<sup>-1</sup>) in the back of the first abdominal segment and another group was injected with sterile saline solution (3% NaCl) as infection control.

After 24 hours, shrimp that had the highest mortality were those of the control group (70%), SGB 9.375g / kg (100%) and SGA 1.25 g / kg (80%) when compared to the other groups. Shrimp fed inclusion level of 2.5 and 3.75 g / kg of SGA and 6.25 g / kg of SGB had the lowest mortalities, being less than 50% in the first 24 hours of challenge. It was observed with the results that the addition of the isoquinoline alkaloids in the feeding of *L. vannamei* increases the survival after challenge with *V. parahaemolyticus*.



**Figure 1:** Cumulative mortality of *Litopenaeus vannamei* fed diets containing different levels of SGA and SGB inclusion after the experimental challenge with *Vibrio parahaemolyticus* at a concentration of  $9 \times 10^7$  CFU mL<sup>-1</sup>.

## REPLACEMENT OF FISHMEAL BY FISH PROTEIN HYDROLYSATE IN FEED FOR POST-LARVAL SHRIMP *Litopenaeus vannamei*

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After filleting of tilapia, the material remaining is discarded and this waste represents about 70% of fish body volume, corresponding to carcass and viscera. These leftovers are important sources of proteins that can be used as feed in aquaculture industry byproducing protein hydrolysates. Thus, the present study aimed to evaluate the zootechnical performance of *Litopenaeus vannamei* fed diets formulated with increasing replacement of fishmeal by two fish protein hydrolysates produced under different hydrolysis times.

Tilapia residues (head, skin, carcass and trimmings) from processing industries were used for FPH production. Two protein hydrolysates were produced, with one (FPH1) and two (FPH2) hours of hydrolysis. The nutritional composition of the hydrolysates showed desirable levels of crude protein (48%) and essential amino acids. Electrophoresis revealed peptides ranging from 10 to 250 kDa. The hydrolysates were incorporated separately in experimental diets in the proportions of 0, 10, 20 and 30%, totalizing 7 diets named 0% (control), H110%, H120%, H130%, H210%, H220%, and H230%. A 45-day feeding trial was carried out to evaluate the zootechnical performance of post-larvae fed these diets.

The regression analysis for final weight indicated the best replacement levels of 1.075% for FPH1 and 15% for FPH2. For survival, the best levels were 11% of FPH1 and 21.5% of FPH2. It can be concluded that the replacement of fishmeal by fish hydrolysate with hydrolysis time of two hours (FPH2) at levels up to 15% promotes better growth of postlarvae of *L. vannamei* without negatively affecting their survival.

## MICROBIOLOGICAL QUALITY OF *Litopenaeus vannamei* CULTURE USING BIOFLOC SYSTEM

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Shrimp farming is a fast-expanding activity that has supported the growth of crustaceans supply to consumers around the world. However, the product is vulnerable to contamination at all stages of the process, including the rearing tanks, where current practices have been increasing stocking densities and reducing water renewal. Therefore, it is important to evaluate the potential of these systems for the proliferation of undesirable microorganisms, which may turn the product unfit for human consumption. In the present study, the presence of coagulase-positive *Staphylococcus*, *Salmonella* spp. and thermotolerant coliforms was verified in biofloc culture systems of *Litopenaeus vannamei* in Brazil, and the results were compared with the legislation regulating the marketing of fresh shrimp.

The *L. vannamei* postlarvae (PL10-12), with a mean weight of  $2.5 \pm 0.5$  mg were stocked (375 shrimp/m<sup>2</sup>) in two tanks (200 m<sup>2</sup> each) with bioflocs. A commercial feed of 40% crude protein (CP) was offered for 20 days four times a day (06:00, 12:00, 18:00 and 00:00 hours). After this period the CP was reduced to 35% and feed was offered twice a day (08:00 and 16:00 hours). The concentration of total ammoniacal nitrogen (TAN) was measured every 2 days to determine the need of additional organic fertilization (molasses). The TAN level was kept below 1 mg /L, using a ratio of 6 g of carbon for each gram of ammoniacal nitrogen in the system. The pH, temperature (°C), salinity and dissolved oxygen concentration (mg/L) were measured twice daily. The volume of bioflocs (mL/L) was evaluated every 3 days. Samples of water and shrimp were collected fortnightly from biofloc tanks for bacteriological analysis, including Coagulase-positive *Staphylococcus*, *Salmonella* spp. and Coliforms. The sampling began on the post-larvae stocking day (week 0) and ending when the shrimp were harvested (week 17).

The bacteriological analyses detected neither coagulase-positive *Staphylococcus* nor *Salmonella* spp. in the samples collected, and this satisfies the norms established by the Executive Council of the Brazilian National Public Health Agency in resolution number 12 of January 2, 2001. The number of thermotolerant coliforms present in the water and shrimp appears to be related to the quantity of bioflocs in the production system. While some samples presented relatively high concentrations of coliforms there are no official criteria for these microorganisms, and all the samples can be considered adequate according to legislation. Despite the high concentration of nutrients available in the bioflocs system, the development of undesirable microorganisms remained controlled and within the limits set by public health agencies, guaranteeing that the final product was fit for human consumption.

## **MINIMIZING THE DRASTIC EFFECTS OF EXPOSURE TO MAXIMUM LEVEL OF PHENOL ON *Oreochromus niloticus* BY USING FEED ADDITIVE ( BIOGEN)**

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In this study diminishes the drastic effects of exposure of *Oreochromus niloticus* to maximum tolerable level of phenol (30ppm) for 10 weeks by using feed additive ( Biogen – China Way Corp. Taiwan) at a ratio of 0.002% were evaluated. The results revealed that, the chronic exposure to 30ppm of phenol significantly decreased the growth performance, survival rate, phagocytic activity, phagocytic index, total serum protein, albumin, globulin and albumin/globulin ratio compared to control of non exposed fish. Moreover, liver function enzymes were significantly increased.

However, addition of Biogen ( 0.2%) to fish feed gave positive results in neutralized the drastic negative effects of phenol on the above mentioned parameters .



# GROWTH PERFORMANCE OF *Clarias gariepinus* (BURCHELL, 1822) FED *Canavalla ensiformis* MEAL AS PARTIAL REPLACEMENT FOR SOYBEAN MEAL

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It is estimated that nutrition in fish farming is about 40 – 60% of the total production cost. (Steven 2001; Craig and Helfrich, 2002; Jamiu and Ayinla, 2003; Tihamiyu *et al.*, 2008). The conventional feedstuffs used in feed formulation are continuously increasing in price because of competitive need from other sectors as well as human consumption (Tihamiyu *et al.*, 2014). In an attempt to reduce the cost of feeding of the African catfish *Clarias gariepinus* (Burchell, 1822) fingerlings, this study was designed to evaluate the nutritional value of raw Jack bean (*Canavalia ensiformis*) seed as partial replacement for expensive soybeans meal. Preliminary investigation was done to compare selected nutritional profile of jack bean and soybeans meal. Result obtained revealed that lysine, histidine, and phenylalanine were significantly higher in *C. ensiformis* compared to the value recorded for soybean meal. However, crude protein and other essential amino acids were significantly lower. Fifty fingerlings of *C. gariepinus* (0.78g±0.03) were then stocked in 15 hapas measuring 1x1x1m<sup>3</sup>, labeled in triplicate according to five iso-nitrogenous diets (35%CP) formulated. Diets were formulated by replacing soybeans meal (58.8% inclusion) with *C. ensiformis* at 10% increment up to 40% with other ingredients fixed as follows; maize meal=26.18%; fish meal=10%; vitamin and mineral premix= 4%; salt and oil= 1%. The result obtained after 56days feeding trials revealed that the fingerlings can tolerate up to 20% replacement without deleterious effect on growth and nutrient utilization. Beyond this, growth was significantly reduced and mortality was increased. It was concluded that dietary inclusion of raw *C. ensiform* meal should not be more than 11% (or 20% replacement for soybeans meal included at 58.8%) in the diet of *C. gariepinus*.

**Table 1: Profile of selected nutrient in raw *C. ensiformis* and soybeans meal**

| Parameters            | Jackbean           | Soybeans           | SEM  |
|-----------------------|--------------------|--------------------|------|
| Amino Acids           |                    |                    |      |
| Lysine                | 6.55 <sup>a</sup>  | 6.41 <sup>b</sup>  | 0.21 |
| Histidine             | 2.64 <sup>a</sup>  | 2.43 <sup>b</sup>  | 0.02 |
| Methionine            | 1.05 <sup>b</sup>  | 1.42 <sup>a</sup>  | 0.17 |
| Threonine             | 3.44 <sup>b</sup>  | 3.91 <sup>a</sup>  | 0.03 |
| Isoleucine            | 3.25 <sup>b</sup>  | 4.64 <sup>a</sup>  | 0.54 |
| Leucine               | 7.03 <sup>b</sup>  | 7.78 <sup>a</sup>  | 0.35 |
| Tyrosine              | 3.12 <sup>b</sup>  | 4.21 <sup>a</sup>  | 0.50 |
| Valine                | 4.21 <sup>b</sup>  | 4.61 <sup>a</sup>  | 0.06 |
| Phenylalanine         | 5.24 <sup>a</sup>  | 4.15 <sup>b</sup>  | 0.23 |
| Cystine               | 1.10 <sup>b</sup>  | 1.68 <sup>a</sup>  | 0.04 |
| Proximate composition |                    |                    |      |
| Moisture              | 7.24 <sup>a</sup>  | 7.07 <sup>b</sup>  | 0.24 |
| Ash                   | 3.88 <sup>b</sup>  | 5.87 <sup>a</sup>  | 1.03 |
| Lipid                 | 5.25 <sup>a</sup>  | 7.03 <sup>b</sup>  | 0.34 |
| Fibre                 | 7.14 <sup>a</sup>  | 4.38 <sup>b</sup>  | 0.09 |
| Protein               | 25.31 <sup>b</sup> | 43.80 <sup>a</sup> | 2.40 |

Mean in the same row with different superscripts differ (P<0.05)

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**Table 2: Nutritional indices of *Clarias gariepinus* (initial weight of  $0.78 \pm 0.03$ ) fed diets containing partially replaced soybeans with *C. ensiformis* meal for 56days.**

|     | DIET 1             | DIET 2             | DIET 3             | DIET 4             | DIET 5             | SEM  |
|-----|--------------------|--------------------|--------------------|--------------------|--------------------|------|
| FW  | 3.81 <sup>a</sup>  | 3.78 <sup>a</sup>  | 3.53 <sup>a</sup>  | 2.66 <sup>b</sup>  | 2.69 <sup>b</sup>  | 0.45 |
| WG  | 3.03 <sup>a</sup>  | 2.98 <sup>ab</sup> | 2.75 <sup>b</sup>  | 1.89 <sup>c</sup>  | 1.89 <sup>c</sup>  | 0.23 |
| GR  | 0.054 <sup>a</sup> | 0.052 <sup>a</sup> | 0.050 <sup>a</sup> | 0.034 <sup>b</sup> | 0.034 <sup>a</sup> | 0.02 |
| SGR | 2.82               | 2.74               | 2.71               | 2.29               | 2.27               | 0.31 |
| FCR | 2.06 <sup>c</sup>  | 2.19 <sup>b</sup>  | 2.21 <sup>b</sup>  | 2.81 <sup>a</sup>  | 2.76 <sup>a</sup>  | 0.32 |
| FER | 44.24 <sup>a</sup> | 43.08 <sup>a</sup> | 42.05 <sup>a</sup> | 36.00 <sup>b</sup> | 37.46 <sup>b</sup> | 2.04 |
| PER | 0.087              | 0.071              | 0.064              | 0.054              | 0.054              | 0.06 |
| SUR | 96.67 <sup>a</sup> | 95.3 <sup>a</sup>  | 94.70 <sup>a</sup> | 76.70 <sup>b</sup> | 66.70 <sup>c</sup> | 2.10 |

Mean in the same row with different superscripts differ ( $P < 0.05$ ).

FW= final weight (g); WG=weight gain (g); GR=growth rate ( $\text{gday}^{-1}$ ); SGR=specific growth rate ( $\%\text{day}^{-1}$ ); FCR= feed conversion ratio; FER= feed efficiency ratio (%); PER= protein efficiency ratio; SUR= survival (%).

## DEPENDENCE OF PH VALUES IN THE DIGESTIVE TRACT OF FRESHWATER FISHES ON SOME ABIOTIC AND BIOTIC FACTORS

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The analysis of the efficiency of fish digestion under natural and aquaculture conditions requires of detailed studies on various aspects of this produces and especially the digestive enzyme complex. It is very well known that the influence of temperature and pH on the activity of digestive enzymes is very strong. Unfortunately, there is still a lot of gaps in our understanding of factors that could affect on the pH in the fish gut.

The goal of the present study was to characterize the values of pH in the digestive tracts of various freshwater fish from different limnetic habitats and to determine the main factors affecting the changes in these values (e.g., seasonal and interannual fluctuations of water temperature, feeding preferences, and level of gastric and intestinal fullness). For this purpose, we have studied 20 freshwater fish species (gastric and agastric) from different Russian water bodies (Lake Teletskoye (51°79'N; 87°26'E), Lake Baikal (52°00'N; 106°11'E), the estuarine area of the Lake Malyie Chany – the Kargat River (54°50'N; 77°40'E) and Kolyma River (69°05'N; 160°08'E)) where there is a large variation in interannual environmental conditions, especially water temperature that greatly varies between seasons. Values of pH were measured within the first 5 to 10 min after euthanasia with a HI 8314 portable pH meter with a HI 1083 B microelectrode of 3 mm of diameter (Hanna Instruments, USA). Depending on the structure of the digestive tract, pH values were measured in the stomach, pyloric caeca and intestine (anterior, middle and posterior intestinal segments).

In the stomachs of studied fish the values of pH varied from strongly acid to neutral; in some fish, weakly alkaline. The variations of pH are not always statistically significant and most likely are dependent neither on the gut length nor on the fish feeding preferences. Only in six out of 20 fish species studied the differences in pH values between the parts of intestine are significant. Such factors as feeding habits of fishes and fullness of their digestive tract affect the values of pH. Temperature is one of the most important factors affecting pH value. This is confirmed by revealed decrease in the intestinal pH values with water temperature in summer time, their lower values in warmer years in the same waterbody and in the waterbodies with higher water temperatures. It is hypothesized that dependence of pH in the fish digestive tract on temperature may serve as a regulatory mechanism for maintaining the activities of hydrolytic enzymes at the level required for successful functioning of digestive system.

## DIGESTIVE ENZYMES OF SIBERIAN SYMPATRIC PAIR OF WHITEFISH (*Coregonus*): FUNCTIONAL AND STRUCTURAL ANALYSIS

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*Coregonus lavaretus* – is widely spread species of whitefish in the north hemisphere that may form different sympatric pairs in lakes. One of such pair inhabits in Lake Teletskoye. The sympatric pair is formed by *Coregonus lavaretus pidshian* with less than 30 gill rakers on the first brachial arch and *Coregonus pravdinellus* that has more 30 (average is 34) ones. *C. l. pidshian* is zoobenthivorous whereas *C. pravdinellus* is zooplanktivorous. Both of them are very important source of animal protein for natives and very interesting model to study the sympatric evolution process. The main aim of the study was to compare activity of digestive enzymes in different parts of fish gut and make their structural identification.

The fish were caught in Lake Teletskoye (51°79'N; 87°26'E, Altai region, Russia) by nets with mesh size 20–40 mm. After their capture, the fish were dissected and the guts were extracted and frozen in liquid nitrogen until analyze. The activities of alpha-amylase, lipase, non-specific esterase, total alkaline proteases, trypsin, chymotrypsin, carboxypeptidase A and B, aminopeptidase and alkaline phosphatase were assayed. For structural identification of proteins the anterior part of intestine was chosen. For separation of proteins the combination of ion-exchange and hydrophobic high pressure liquid chromatography was conducted and then the collected peaks were run on SDS-PAGE electrophoresis. Then, all obtained bands of protein were transported on PVDF Immobilon SQ membrane and the sequencing of N-terminal aminoacid (15 residues) was done.

The activity of all studied enzymes had the similar trends throughout the all parts of intestine of *C. l. pidshian* and *C. pravdinellus* but the level of activities was different. Thus, the activities of trypsin, chymotrypsin, carboxypeptidase A, alpha-amylase were higher in anterior and middle intestine of *C. l. pidshian*. We extracted and determined the following enzymes: trypsin-, chymotrypsin- and subtilisin-like enzymes, phospholipase like and leucine-aminopeptidase.

## THE ROLE OF AQUACULTURE IN RURAL DEVELOPMENT

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Rural development, the process of sustained growth of the rural economy and improvement of well-being of rural men, women and children, has various dimensions, but it is particularly the development of the agricultural sector, which is widely believed to provide the main impetus not only for reducing poverty and hunger but also for ensuring food security for all. Only if more rapid agricultural growth takes place in countries with impoverished rural populations, can rural farm and non-farm incomes rise sufficiently to enable the rural poor to become more food secure.

Various types of aquaculture form an important component within agricultural and farming systems development. These can contribute to the alleviation of food insecurity, malnutrition and poverty through the provision of food of high nutritional value, income and employment generation, decreased risk of monoculture production failure, improved access to water, enhanced aquatic resource management and increased farm sustainability (e.g. FAO 2013a, Prein and Ahmed 2014).

Global aquaculture is now the fastest growing food production sub-sector in many countries. The production of all cultured aquatic organisms reached almost 43 million metric tonnes (mmt) in 2013 (FAO 2015), and it is expected that this trend will continue despite several constraints, which may become more challenging in the future.

FAO supports this process by promoting sustainable aquaculture development in its member countries and aims to assist them in achieving an increased contribution of this sector to rural development.

The purpose of this paper is to analyse the role of aquaculture in rural development, through its relationship to food security and poverty alleviation, its contribution to rural development, and to indicate strategies that could increase this contribution. It covers both inland areas and coastal zones and has no distinct geographical focus. However, the overall emphasis is on developing countries, which are the source of over 80% of world aquaculture production and where almost 75% of the poor live in rural areas.

## NEW DEVELOPMENTS IN RECIRCULATION AQUACULTURE SYSTEMS IN EUROPE: A PERSPECTIVE ON ENVIRONMENTAL SUSTAINABILITY

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The dual objective of sustainable aquaculture, i.e., to produce food while sustaining natural resources is achieved only when production systems with a minimum ecological impact are used. Recirculation aquaculture systems (RASs) provide opportunities to reduce water usage and to improve waste management and nutrient recycling. RAS makes intensive fish production compatible with environmental sustainability. This review aims to summarize the most recent developments within RAS that have contributed to the environmental sustainability of the European aquaculture sector. The review first shows the ongoing expansion of RAS production by species and country in Europe. Life cycle analysis showed that feed, fish production and waste and energy are the principal components explaining the ecological impact of RAS. Ongoing developments in RAS show two trends focusing on:

(1) technical improvements within the recirculation loop and (2) recycling of nutrients through integrated farming. Both trends contributed to improvements in the environmental sustainability of RAS. Developments within the recirculation loop that are reviewed are the introduction of denitrification reactors, sludge thickening technologies and the use of ozone. New approaches towards integrated systems include the incorporation of wetlands and algal controlled systems in RAS. Finally, the review identifies the key research priorities that will contribute to the future reduction of the ecological impact of RAS. Possible future breakthroughs in the fields of waste production and removal might further enhance the sustainability of fish production in RAS.

**Abbreviations:** RASs, recirculating aquaculture systems; LCA, life cycle analysis; ISO, International Organization for Standardization; GWP, global warming potential; NPPU, net primary product use; NPP, net primary product; EP, eutrophication potential;  $Eu$ , energy use; AP, acidification potential; FTS, flow-through systems; FCR, feed conversion ratio; IMTA, integrated multi-trophic aquaculture; USBR, upflow sludge blanket reactor; HRAP, high-rate algal ponds; PAS, partitioned aquaculture systems; Anammox, anaerobic ammonium-oxidation; TOD, total oxygen demand; COD, chemical oxygen demand; BOD, biological oxygen demand; TSS, total suspended solids; TDS, total dissolved solids; TN, total nitrogen; TAN, total ammonia nitrogen; TP, total phosphorus; OC, organic carbon

### Introduction

Aquaculture has been on the frontline of public concerns regarding sustainability. Different issues are raised, such as the use of fish meal and oil as feed ingredients (Naylor et al., 2000), escapees of farmed fish from sea cages into the wild and the discharge of waste into the environment (Buschmann et al., 2006). Recirculation aquaculture systems (RAS) are systems in which water is (partially) reused after undergoing treatment (Rosenthal et al., 1986). Each treatment step reduces the system water exchange to the needs of the next limiting waste component. Based on system water exchange it is possible to distinguish between flow through ( $>50 \text{ m}^3/\text{kg feed}$ ), reuse ( $1\text{--}50 \text{ m}^3/\text{kg feed}$ ), conventional recirculation ( $0.1\text{--}1 \text{ m}^3/\text{kg feed}$ ) and 'next generation' or 'innovative' RAS ( $<0.1 \text{ m}^3/\text{kg feed}$ ). RAS have been developed to respond to the increasing environmental restrictions in countries with limited access to land and water. Furthermore, the new EU water management directive (Directive 2000/60/EC 23rd Oct 2000) calls for sound environmental friendly aquaculture production systems. RAS offer advantages in terms of reduced water consumption (Verdegem et al., 2006), improved opportunities for waste management and nutrient recycling (Piedrahita, 2003) and for a better hygiene and disease management (e.g. Summerfelt et al., 2009; Tal et al., 2009), biological pollution control (no escapees, Zohar et al., 2005), and reduction of visual impact of the farm. These systems are sometimes referred to as 'indoor' or 'urban' aquaculture reflecting its independency of surface water to produce aquatic organisms. In addition, the application of RAS technology enables the production of a diverse range of (also exotic) seafood products in close proximity to markets (Masser et al., 1999; Schneider et al. 2010), thereby reducing carbon dioxide ( $\text{CO}_2$ ) emissions associated with food transport and the negative trade deficits related to EU imports of seafood.

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Despite its environmentally friendly characteristics and the increasing number of European countries applying RAS technology, its contribution to production is still small compared to (sea) cages, flow-through systems or ponds. The slow adoption of RAS technology is in part due to the high initial capital investments required by RAS (Schneider et al., 2006). High stocking densities and productions are required to be able to cover investment costs. As a consequence welfare concerns may arise (Martins et al., 2005). However, due to the possibility to maintain a constant water quality, RAS may also contribute to an improved welfare (Roque d'Orbcastel et al., 2009a).

Managing disease outbreaks pose specific challenges in RAS in which a healthy microbial community contributes to water purification and water quality. Minerals, drug residues, hazardous feed compounds and metabolites may accumulate in the system (Martins et al. 2009a,b) and affect the health, quality and safety of the farmed animal. How these different factors interact and influence the fish and the various purification reactors is still poorly understood. Furthermore, RAS historically developed producing freshwater fish species that are rather tolerant to poor water quality. The expansion of RAS being used for the production of marine and brackish water species often focuses on hatchery operations which pose extra requirements on water quality and require further innovations in RAS technology.

Taken together, these examples reflect environmental, economic and social challenges to the sustainability of RAS. Considering these challenges, an European effort was made (e.g. CONSENSUS, [www.euraquaculture.info/](http://www.euraquaculture.info/), SUSTAINAQUA, [www.sustainaqua.com](http://www.sustainaqua.com); SUSTAINAQ [www.sustainaq.net](http://www.sustainaq.net); AQUAETREAT [www.aquaetreat.org](http://www.aquaetreat.org)) to identify the most relevant sustainability issues for RAS, to quantify sustainability in RAS and to develop new technologies to improve sustainability of RAS. This review summarizes recent developments that contributed to the environmental sustainability of the aquaculture production in RAS in Europe. These developments are either technology (e.g. incorporation of new water treatment units that reduce water exchange rates and reduce/concentrate waste) or ecology driven (e.g. biological re-utilisation of wastes).

#### RAS within European Aquaculture- Representative species, countries and production

Data on RAS production is generally difficult to evaluate, as there is no compiled dataset available for this type of production system in Europe. For the purpose of this review an update of the previously published production data in RAS (Martins et al., 2005) was performed. Nevertheless, both grow-out and fingerling production data are still incomplete and many national organizations and stakeholders are not able to provide conclusive data. Tables 1 and 2 summarize the updated production data by species and country. RAS technology is mainly established in The Netherlands and Denmark with raising interest in other European countries for both hatchery and grow-out production. The Dutch RAS are typically indoor, nearly closed systems (water refreshment rate ranges between 30L/kg feed/day and 300L/kg feed/day, Martins et al., 2009b) for freshwater production of African catfish and eel. The Danish model trout farms are outdoor, semi-closed systems for trout on- growing using 3900 L/kg feed or 1/13 of traditional trout farming (Jokumsen et al., 2009). In France a trout RAS, designed after the Danish model trout farms was operated at a water refreshment rate of 9000 L/Kg feed/day (Roque d'Orbcastel et al., 2009b).

Since their introduction in the late 80's RAS production increased significantly in volume and species diversity (Rosenthal, 1980; Verreth and Eding, 1993; Martins et al., 2005). Today more than 10 species are produced in RAS (African catfish, eel and trout as major freshwater species and turbot, seabass and sole as major marine species). Recently, new facilities were established in the UK (sea bass), France (salmon) and Germany (different marine species). Two major new developments in Europe at the moment are the increasing production of trout in outdoor RAS in Denmark and the decreasing production of African catfish and European eel in indoor RAS in The Netherlands. In Denmark, government pressure and rules limiting feeding stimulated the shift towards outdoor RAS (Pedersen et al., 2008). Competition with *Pangasius* imports for African catfish and increasing societal pressure to reduce consumption of endangered eel constraints the demand (van Duijn et al., 2010). At the moment there is serious doubt if the Dutch production for these two species will be able to recover to former levels or stay at this lower production volume.

Available data suggests that hatchery production is shifting towards RAS technology. An example is the production of Atlantic salmon smolts in the Faeroe Islands where a complete shifting from flow-through farms into RAS took place after 2000 (Bergheim et al., 2008, 2009). Joensen (2008) reported an increase of smolt size from 50-70 g from flow-through farms to 140-170 g in RAS. In Norway a production of 85 million smolts in RAS is foreseen (Del campo et al., 2010). Future water shortage, large season variation in water temperature and low inlet water quality (including aluminium concentrations) are the main factors driving the shift of smolts production from flow-through to RAS in Norway (Kristensen et al., 2009). In addition, Terjesen et al. (2008) suggested an increased smolt quality (growth and survival after sea transfer) in RAS- cultured smolts.

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## New developments in RAS leading to environmental sustainability

### Sustainability assessment: Is RAS environmentally sustainable?

Life Cycle Analysis (LCA) is an International standardized method (ISO, 2006) designed to assess the global and regional impacts of a product or a process on the environment. It implies impact assessment of all actions and means required to produce, distribute and use a product: raw material use, infrastructures, energy, processing and all the emissions (in air, water and soil) linked to the product or process. The LCA can be divided into four steps: definition of the system limits, data inventory, data translation into environmental impact indicators and results analysis and interpretation.

LCA has been used to study the environmental sustainability of aquaculture systems (Seppala et al., 2001; Papatryphon et al., 2004a,b; Aubin et al., 2006, 2009; Ayer and Tyedmers, 2009; Ellingsen et al., 2009; Roque d'Orbcastel et al., 2009c). Environmental impact indicators are defined both at the global and at the regional levels. Indicators usually used for fish farms are, at the global level, the Global Warming Potential (GWP in kg CO<sub>2</sub> eq.) which measures the impact of gaseous emissions as CO<sub>2</sub>, methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O) on global greenhouse effect, the Energy use (E in MJ) which corresponds to all energy

sources (coal, gas, uranium, etc) used in the system, the surface use (m<sup>2</sup>) which represents the land surface used in the system life cycle and sometimes the Net Primary Product Use (NPPU in kg of carbon (C)) which represents the use of net primary product (NPP) as a biotic 3-resource. At the regional level, the Eutrophication Potential (EP in kg PO<sub>4</sub> 3-equivalent or PO<sub>4</sub> eq) measures the environmental impact of macronutrients such as nitrogen and phosphorus on ecosystems and the Acidification Potential (AP in kg SO equivalent or SO eq) evaluates the impact of acidifying pollutants (sulphur dioxide, SO<sub>2</sub>; ammonia, NH<sub>3</sub>; nitrite, NO<sub>2</sub>; nitrogen oxides, NO<sub>x</sub>) on ecosystems.

Using LCA, Roque d'Orbcastel et al. (2009c) compared the environmental impacts of 3 systems of which 2 RAS and one flow-through system (FTS) (Fig. 1).

Contribution analysis showed that in FTS and RAS, **Feed** had the strongest impact on all indicators, **Fish production and wastes** explained 50 to 60% of the system's eutrophication potential and Energy use was mainly due to **Electricity** consumption to operate the systems (2/3 in RAS and 1/2 in FTS) and feed (1/3 in RAS and 1/2 in FTS). Other contribution categories explained less than 6.5 % of the global environmental impact (4 % for equipments, less than 2 % for infrastructures and less than 0.2 % for chemicals).

### Feed

First solution to reduce the environmental impacts of aquaculture systems consists in minimizing the Feed Conversion Ratio (FCR): a 30% reduction of FCR in a trout farm resulted in a reduction of almost 20% of the global environmental impact, excluding energy use (Roque d'Orbcastel et al., 2009c). RAS provides optimal environmental conditions all year round (total ammonia nitrogen and dissolved CO<sub>2</sub> concentrations were lower in the RAS than in the FTS), contributes to fish welfare and minimizes the FCR, hence improving feed efficiency (Losordo 1998a; Losordo 1998b; Roque d'Orbcastel et al., 2009a). Feed impact on the environment may also be reduced by choosing local feed ingredients and ingredients from a low trophic level (e.g. proteins and lipids from phytoplankton rather than from fish), provided feed digestibility does not decrease.

### Fish Production and Waste

High flow rates of low concentrated effluents are the main obstacle to the economic treatment of waste water from FTS. By comparison, the flow rate of RAS waste water is 10 to 100 times lower and 10 to 100 times more concentrated (Blancheton et al., 2007), which allows for easier and more cost effective treatment.

Pedersen et al. (2008) also showed a reduction on the environmental impact from converting flow through trout farms into RAS including waste management. In RAS, removal efficiencies were between 85 – 98 % for organic matter and suspended solids and between 65 – 96 % for phosphorous.

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Different combinations of waste treatment systems were studied at marine and freshwater fish farms operated in flow through or in recirculation, through an EU project ([www.aquaEtreat.org](http://www.aquaEtreat.org)). The general treatment scheme implemented at all the farms included a series of water treatment units at different locations in the farms and settling of backwash water, to obtain (1) sludge with more than 15% of dry matter, which may be valorised as fertilizer directly or after composting, (2) supernatant water from the backwash water tank, that can be treated through constructed wetlands alleviating the load of suspended solids and the biological oxygen demand (Roque d'Orbcastel, 2008) and (3) filtered water (recirculating water low in suspended solids) which returns to the fish tanks. Most of the time, filtered water from flow through systems is not treated. However, according to the fish biomass, water flow rate and legislation, total ammonia nitrogen (TAN) concentration can reach levels requiring a treatment. It is a true economic challenge as the water flow rate to be treated is high (50 to 100 m<sup>3</sup> / kg fish produced) while the nutrient concentrations in the effluent are low (e.g. around 1 mg TAN/L). Concerning marine RAS, filtered water may be treated in a High Rate Algal Pond (HRAP) (see latter section 3.3) and reused in RAS without inducing sea bass mortality or decreasing growth and reducing the water consumption to less than 1 m<sup>3</sup> of water per kg fish produced (Metaxa et al., 2006). Improved waste treatment and linkage with cultures of extractive species may further alleviate the environmental impact from fish farms. Integrated Multi-Trophic Aquaculture (IMTA) where the by-products (wastes) from one species become inputs for other co-cultured species (Hussenot, 2006) may be the solution.

### Energy

Roque d'Orbcastel et al. (2009c) calculated that energy use through LCA is 1.4–1.8 higher in RAS (63,202 MJ per ton of fish or 16 kWh per kg fish) than in flow-through systems. Energy use reduction in RAS is possible by improving the system design and management of airlifts and biofilters (Roque d'Orbcastel et al., 2009c) or the incorporation of denitrification in the recycling loop (Eding et al., 2009). A reduction of transport of feed ingredients in fish feeds will further lower energy consumption.

Table 3 shows that the energy consumption per kg of trout or sea bass produced in FTS and RAS is comparable to the amount needed to capture 1 kg of cod at sea (5 to 21 kW/kg). Recent RAS designs minimize height differences between RAS compartments and also pumps became more efficient or replaced by air lifts. This resulted already in a 50% reduction in energy use, a trend which continues, considering further improvements such as completely low head RAS with only few centimetres of height differences or raceway systems that use and treat water alongside cascades.

### Developments in the recirculation loop

Producing fish in conventional RAS, in which a large volume of water is refreshed and a limited number of water treatments units are used (essentially mechanical waste removal and biofiltration) has a smaller environmental impact than flow through systems. Recent innovations such as denitrification reactors, sludge thickening technologies and ozone treatments led to a further decrease in water use, waste discharge and energy use in RAS. In addition, the discharged waste is more concentrated, facilitating waste (re-)use options as fertilizer or in integrated, eventually completely closed, systems (reviewed in section 3.3). Combined, these developments certainly improve the environmental sustainability of RAS.

### Denitrification reactors

Conventional RAS are operated at variable water refreshment rates (0.1-1 m<sup>3</sup>/kg feed). For instance in RAS producing European eel, refreshment rates are about 200-300L per kg feed (Eding and Kamstra, 2002; Martins et al., 2009b). In these systems, solids are removed by sedimentation or sieving, oxygen is added by aeration or oxygenation, carbon dioxide is removed by degassing and ammonia is mostly converted into nitrate (NO<sub>3</sub>) through nitrification in aerobic biological filters. In a conventional RAS the maximum allowed concentration of NO<sub>3</sub> steers the external water exchange rate (e.g. Schuster and Stelz, 1998). High nitrate concentrations can be counteracted by denitrification (Rijn and Rivera, 1990; Barak, 1998; Rijn and Barak, 1998; van Rijn et al., 2006). Denitrification reactors applied to RAS have different designs (see review from van Rijn et al., 2006). One of the designs that have been used successfully in pilot scale recirculating systems is the upflow sludge blanket denitrification reactor (USB-denitrification reactor, Figure 2). This reactor is a cylindric anoxic (no free dissolved oxygen; NO<sub>x</sub> present) reactor fed with dissolved and particulate faecal organic waste, bacterial flocs and inorganic compounds trapped by the solids removal unit. The waste flow enters the reactor at the bottom centre. The up flow velocity in the reactor is designed to be smaller than the settling velocity of the major fraction of the particulate waste in order to create a sludge bed at the bottom. In the sludge bed the faecal particulate waste is digested by the denitrifying bacteria and results in: (1) the production of bacterial biomass,

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(2) reduction of NO<sub>3</sub> into nitrogen gas (N<sub>2</sub>), (3) CO<sub>2</sub> release, and (4) alkalinity and (5) heat production. The particulate waste in the sludge bed serves also as substrate for the denitrifying bacteria. Pre-settled water leaves the reactor through a V-shaped dented overflow at the top section of the reactor.

As an example, since 2005, a denitrification reactor using internal carbon source, was integrated into a conventional RAS (Figure 2) in The Netherlands. In a 600 MT/year Nile tilapia *Oreochromis niloticus* RAS farm the water exchange rate was as low as 30 L/kg feed, corresponding to 99% recirculation (Martins et al., 2009b). Such an extreme low water exchange rate became possible by incorporating a denitrification reactor in RAS to convert NO<sub>3</sub> into nitrogen gas (N<sub>2</sub>). Organic matter (either of external origin, i.e. methanol, but preferably of internal origin, i.e. the uneaten feed and faeces from the solids removal) is oxidized by reducing NO<sub>3</sub>. Compared to a conventional RAS, this latest generation RAS thus reduce water consumption, and NO<sub>3</sub> and organic matter discharge. The costs for installation and operation of the denitrification reactor are outweighed by the reduction in costs for discharge to the local sewer, groundwater permits restricting groundwater extraction at one production location and the increasing energy costs for heating groundwater to 28 °C (Martins et al., 2009b). Considering the nutrient balance before and after on-farm implementation of denitrification on the hypothetical 100 MT/y tilapia farm mentioned before (Eding et al., 2009), performance of a 100 MT/y tilapia RAS with and without denitrification was compared for the sustainability parameters nutrient utilization efficiency (%), resource use and waste discharge per kg fish produced (Table 4). It can be seen that the RAS with denitrification has substantially lower requirements for heat, water and bicarbonate. Although the RAS with denitrification has somewhat higher requirements for electricity, oxygen and labour (and investments), the actual production costs per kg harvested fish are approximately 10% lower than for the conventional RAS. Waste discharge is reduced by integration of denitrification by 81% for nitrogen (N), 59 % for chemical oxygen demand (COD), 61% for total oxygen demand (TOD), 30% for CO<sub>2</sub> and 58% for total dissolved solids (TDS). Integrating a USB-denitrification reactor in a conventional RAS allows to (1) reduce the make-up water volume necessary for NO<sub>3</sub> control, (2) reduce NO<sub>2</sub> discharge, (3) reduce energy consumption due to heat production by the bacterial biomass in the reactor and a reduction in the volume of make-up water that needs to be heated, (4) concentrate and reduce the drum filter solids flow, by digesting the solids in situ, reducing fees for discharge of TAN, NO<sub>3</sub>, organic nitrogen, and organic matter (measured as COD), and (5) increase alkalinity allowing a pH neutral fish culture operation. Kim and Bae, 2000 E.W. Kim and J.H. Bae, Alkalinity requirements and the possibility of simultaneous heterotrophic denitrification during sulfur utilizing autotrophic denitrification, Water Sci. Tech. 42 (2000), pp. 233–238. View Record in Scopus | Cited By in Scopus (24).

Despite the considerable advantages of introducing a denitrification reactor in a conventional RAS, its use in commercial farming is still limited. Major reasons include the higher investments, the required expertise and the accumulation of TDS on farm or the alternative use of an external carbon source. In most EU countries, the economical feasibility of using a denitrification reactor still has to be demonstrated.

One of its major contributions to environmental sustainability of integrating denitrification in RAS is the reduction in water use. However, a small water exchange rate might also create problems. As pointed out by Martins et al (2009 a,b) such reduction may lead to an accumulation of growth inhibiting factors originating from the fish (e.g. cortisol), bacteria (metabolites) and feed (metals). Using a bioassay, Martins et al. (2009a) showed that with a low water exchange of 30L per kg feed, the accumulation of phosphate (PO<sub>4</sub>), NO<sub>3</sub> and of the heavy metals arsenic and copper is likely to impair the embryonic and larval development of common carp and therefore deserves further research. Also, Davidson et al. (2009) suggested a negative impact on survival of reducing water refreshment rates in trout cultured in RAS, mainly due to the accumulation of copper. Nevertheless, in grow out, Good et al. (2009) and Martins et al. (2009b) showed no impact on growth performance of fish cultured in low water exchange RAS. In turbot RAS no growth retardation could be detected compared to re-use of flow through systems during long term experiments (about 550 days) running those systems under commercial conditions (Schram et al., 2009)

#### Sludge thickening technologies

Sludge discharge from RAS requires storage facilities, transportation, labour and disposal fees (Schneider et al., 2006). Thickening technologies such as belt filter systems (Ebeling et al., 2006) and geotextile bags or tubes (Ebeling et al., 2005; Sharrer et al., 2009) can decrease this problem. These systems allow a dewatering of the sludge and therefore a reduction in the volume of solids produced.

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Sharrer et al. (2009) suggested that using geotextile bag filters in RAS provide an excellent pretreatment in situations where the total suspended solids (TSS) must be dewatered before disposal, because 1) leaching of dissolved organic carbon and COD from this waste is desired to drive denitrification or 2) leaching of inorganic nitrogen and PO<sub>4</sub> from the waste is desired to feed nutrients to downstream hydroponic operations or field crops (Ebeling et al., 2006). In addition, when geotextile tubes are incorporated in a RAS + denitrification reactor system, the solids waste volume could be concentrated to a dry matter content of 9.1% after 7 days of dewatering when supplying polymer as coagulation/flocculation aid to the weekly discharged sludge from the denitrification reactor (Eding et al., 2009). However, results within the scope of the AquaEtrete project (<http://www.aquaetreat.org>) showed that the use of polymer for trout sludge thickening was too expensive for ensuring sustainable production in France and Italy.

Phosphorus is one of the nutrients contributing most to the eutrophication of waters receiving effluents from intensive aquaculture. Therefore, any reduction in phosphorus levels in aquaculture effluents will improve the environmental sustainability of RAS. Targeting to further improve the solid removal efficiency from RAS is a logical first step as the filterable or settleable solids fractions of aquaculture effluents contain the highest fraction of discharged P (Heinen et al., 1996). Rishel and Ebeling (2006) using a combination of alum/polymer in a flocculation unit obtained removal rates > 90% for TSS, PO<sub>4</sub>, total phosphorus (TP), biological oxygen demand (BOD) and COD from aquaculture effluents. These authors also showed an effect of the coagulation/flocculation aids on the nitrogen removal: TAN, NO<sub>3</sub>, NO<sub>2</sub>, and total nitrogen (TN) in the wastewater effluent were reduced on average by 64, 50, 68, and 87%, respectively.

#### Ozone

Ozone has been used in RAS to control pathogens (e.g. Bullock et al., 1997) and to oxidize NO<sub>2</sub> to NO<sub>3</sub>, organic matter, TAN, or fine suspended particles (e.g. Tango and Gagnon, 2003; Summerfelt et al., 2009). Ozonation improves microscreen filter performance and minimizes the accumulation of dissolved matter affecting the water colour (Summerfelt et al., 2009). Generally a wide range is referred in literature, 3- 24 g ozone for every kg of feed to a RAS, to sustain good water quality and fish health (Bullock et al., 1997; Summerfelt, 2003).

However, ozonation by-products could be harmful. Bromate is one of such by-products and potentially toxic. Tango and Gagnon (2003) showed that ozonated marine RAS have concentrations of bromate that are likely to impair fish health. Therefore, the consequences to the fish of applying ozone in RAS should be further investigated.

#### New approaches towards integrated systems

Although strictly spoken, a RAS should minimally contain one fish tank and one water treatment unit, sometimes a stagnant aquaculture pond is referred to as a single reactor RAS. All processes managed in separate reactors in RAS also occur in ponds: algae or macrophyte production, sedimentation, nitrification, denitrification, acidification, phosphate precipitation, aerobic and anaerobic decomposition, fish production, heating or cooling, etc. By compartmentalizing some of these processes in reactors besides the fish tank the total production capacity of the system is increased (Verdegem et al., 1999; Schneider et al., 2005; Gál et al., 2007). However the overall treatment efficiency using especially phototrophic reactors is currently still too low and leads to a mismatch in surface areas between fish production and phototrophic reactor by at least one magnitude (Schneider et al., 2002). The re-use of this biomass as feed is again decreasing the overall efficiency of the treatment process by 90%.

Recently, wetlands and algal ponds received a lot of attention as water treatment units in RAS, as they contribute to the water reuse in the system.

#### Wetlands

Effluents from fish tanks, ponds or raceways are 20-25 times more diluted than medium strength municipal wastewater commonly treated in constructed wetlands (Vymazal, 2009). Wetlands are mostly used to treat aquaculture effluents after concentrating the wastes, at which point they are considered a low cost and viable biological treatment method (Sipaúba-Tavares and Braga, 2008). Kerepeczki et al. (2003) directly treated the effluent from an intensive African catfish operation, passing the effluent first through carp ponds and subsequently through ponds converted into wetland. In this pond-wetland system, removal rates above 90% were obtained for TAN, PO<sub>4</sub> and organic suspended solids and between 65 and 80% for inorganic nitrogen compounds, TN and TP. The removal rate of NO<sub>3</sub> was 38%. Most constructed wetlands used in aquaculture are soil based horizontal subsurface flow systems. Reviewing 20 years operation of this type of constructed wetlands in Denmark, Brix et al. (2007) concluded that the BOD and organic matter reduction is excellent, but that the

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removal of N and P is typically only 30-50%. In addition, nearly no nitrification occurs in these horizontal subsurface flow systems. To reduce the TAN concentration in the effluent to  $< 2$  mg/L, a fixed film aerated nitrification filter needed to be added. In recent years, to improve TAN and  $\text{NO}_3$  removal, newly installed systems are vertical flow constructed wetlands with partial recirculation. Partial recirculation of the effluent stabilizes system performance, and enhances nitrogen removal by denitrification (Arias et al., 2005). Nevertheless, Summerfelt et al. (1999) compared a vertical and horizontal flow constructed wetland to treat the concentrated solids (5% dry matter) discharge from a trout farm. The vertical flow wetland performed better for total COD and dissolved COD removal, but both type of wetlands performed equally well for total Kjeldahl nitrogen, TP and  $\text{PO}_4$  removal. Apparently, numerous factors influence the performance of constructed wetlands for effluent treatment. Plant species and sediment type are important in determining the treatment efficiency of constructed wetlands. Rhizome forming plants are less efficient in removing TAN and  $\text{NO}_3$  than plants forming fibrous roots (Chen et al., 2009). Plants mainly affect the removal of organic matter and N species, while sediments like steel slag or limestone are excellent for P removal (Naylor et al., 2003). Testing different combinations of plant species and sediment types to treat a fish farm effluent from an anaerobic digester, it was impossible to maximize in one step simultaneous removal of organic matter, nitrogen and phosphorous. The recommendation was given to use two sequential units, first a macrophyte planted basin with a neutral substrate, followed by a plant-free basin with a phosphorous absorbing substrate. A similar approach was followed by Comeau et al. (2001) to treat the effluent from a  $60 \mu\text{m}$  screen drum filter on a trout farm. By passing the effluent first through a plant bed, then through a phosphorous removing bed more than 80% of the TP mass load and 95% of the suspended solids were removed.

The nutrient removal efficiency in constructed wetlands of non-concentrated aquaculture effluents tends to be lower than for concentrated effluents. On average, 68% of COD, 58% TP and 30% of TN were removed from trout raceway effluents in a constructed wetland, applying a hydraulic retention time of 7.5 h (Schulz et al. 2003). In a recent study, Sindilariu et al. (2009a) removed up to 75-86% of TAN, BOD<sub>5</sub> and TSS with a uptake of 2.1-4.5 g TAN and 30-98 g TSS/m<sup>2</sup>/d, from trout raceway effluents. With a cost of € 0.20/kg fish, which is less than 10% of the total production costs, subsurface flow constructed wetlands to treat trout farm effluents are considered commercially viable.

Reports of integration of constructed wetlands in partially recirculating fish farms in Europe are rare (Andreasen, 2003; Summerfelt et al., 2004). Water re-use involves costs for pumping and aeration or oxygenation. Advantages include more fish produced per m<sup>3</sup> of water entering the farm and the possibility to remove and concentrate solids from the recirculating flow. In a commercial trout farm, the farm effluent returning to the brook from where it was taken was only enriched with 0.03 mg/L TP, 1.09 mg/L BOD<sub>5</sub> and 0.57 mg/L TSS (Sindilariu et al., 2009b). To achieve this, a combination of screen filtration and extraction of sludge for agriculture manure application in a cone settler was used. The supernatant from the cone settler was led through a subsurface constructed wetland prior to discharge. On average, 64% of the particulate matter, 92% of  $\text{NO}_2$  and 81% of  $\text{NO}_3$  were removed in the constructed wetland.

#### Algal controlled systems

##### *Micro-algae availability*

Aquaculture ponds are eutrophic with a primary production of 1 – 3 g C/m<sup>2</sup>/d in temperate regions and 4-8 g C/m<sup>2</sup>/d in the tropics and subtropics. Nearly all algae are mineralized within the pond. In addition, aquafeeds also act as a fertilizer. If the total primary production would constantly be harvested from ponds, the amount of fertilizer needed to maintain the productivity would be prohibitively high. Pond management aims to maintain production and consumption in equilibrium. Nevertheless, even if only a few % of the primary production could be harvested and used as feed or biofuel (Cadoret and Bernard, 2008), the impact on the biobased economy would be significant. Direct harvesting of algae is difficult. New techniques like flocculation maybe will lead to a breakthrough (Lee et al., 2009).

##### *Micro-algae based water treatment*

Microalgae are used in waste water treatment, supporting the removal of COD and BOD, nutrients, heavy metals and pathogens, and anaerobic digestion of algal-bacterial biomass can produce biogas (Muñoz and Guieysse, 2006). Also dissolved aquaculture wastes can be processed in algal ponds. In turn, the produced algal biomass represents a food resource for a selected number of aquatic species. Wang (2003) reported on a commercial integrated shrimp – algae – oyster culture in Hawaii with reduced water consumption that turns effluent treatment into a profit. The farmer was able to maintain

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a relatively pure outdoor culture of *Chaetoceros* sp. as food for the oyster *Crassostrea virginica*. A major difficulty is to maintain the balance between shrimp, algae and oyster populations. Constant filter feeding by the oysters on *Chaetoceros* is necessary to keep the algae population healthy. A high concentration of *Chaetoceros* helps in reducing pathogens like *Vibrio vulnificus* for the shrimp. Other systems utilizing phototrophic conversions have been summarized and compared in Schneider et al. (2005).

High-rate algal ponds (HRAP) have been designed to match the production of algae and O<sub>2</sub> with the BOD of the influent (Oswald, 1988). HRAPs can remove up to 175 g BOD/m<sup>3</sup>/d, compared to 5-10 g BOD for normal (waste stabilization) ponds (Racault and Boutin, 2005).

A slightly modified concept of HRAPs has been applied for waste treatment in partitioned aquaculture systems (PAS) (Brune et al., 2003). American catfish production is concentrated in raceways in a small fraction of the pond, from where the water passes through a sedimentation basin and subsequently through a shallow algal raceway. Nile tilapias are stocked in the algal section to reduce the algal density. The tilapias filter algae from the water column, reduce the prevalence of blue green algae increasing the presence of green algae, and trap algae in fecal pellets that are easily removed from the water column. Considerable more American catfish is produced in PAS per unit surface area than in conventional ponds. Fine tuning the oxygen dynamics in the systems requires continuous monitoring and highly skilled management, constraining large scale adaptation of PAS technology.

In France, a HRAP was incorporated in a sea bass RAS as a secondary waste water treatment to reduce the discharge of nutrients from the system (Deviller et al., 2004; Metaxa et al., 2006) and reuse the waste water into the RAS. Fish growth was similar in RAS with and without reuse of the water purified in the HRAP. The HRAP treated water had limited effect on the overall functioning of the RAS, but survival was better in the RAS+HRAP system. The concentration of inorganic nitrogen and phosphorous was less in the rearing water of the RAS+HRAP system, while the accumulation of metals in muscle and liver of the sea bass was reduced, except for chromium and arsenic.

Open pond sea bass, sea bream and turbot production units were developed in previous salt ponds along the Atlantic coast in Europe. The continuous culture of microalgae using pond effluents is possible with the continuous addition of the limiting nutrients silicon and phosphorus to obtain a 10N:5Si:1P ratio (Hussenot et al., 1998; Hussenot, 2003). When the hydraulic retention time is adjusted to the temperature dependent growth rate of the algae, 67% of TAN and 47% PO<sub>4</sub> can be removed. For intensive hatchery-nursery systems, in-pond submerged foam fractionation was used, effectively removing dissolved organic carbon and bacteria, and to a lesser extend chlorophyll and PO<sub>4</sub>. The foam fractionation works well in low water exchange ponds, but is not effective in flow-through systems.

#### Looking ahead: priorities for future research

The basic RAS technology seems quite out-engineered, yet, there are many technical innovations needed to enable the systems performing well for a broader range of animals, culture conditions and life stages. Current engineering innovations search for more energy and cost efficient systems, more closed systems, and/or for a cradle-to-cradle approach in system development, whereby wastes are re-used for other purposes or product commodities. Automation, robotisation, and cybernetic control systems are still far from being commonly used but could provide breakthrough innovations. Next to this pure engineering approach, it is envisaged that major breakthroughs have to come from a better understanding of how the animals interact with the RAS biotope. Such understanding may contribute to minimize even further the ecological impact of RAS.

The major area of research that we foresee as priority to improve the ecological sustainability of RAS is the efficiency of waste removal (solids, nitrogen, phosphate) in the system.

#### Solids

Current RAS systems are reasonably well designed to manage nitrogenous wastes and gaseous exchange, but not to manage solid wastes. The main bottleneck is related in the fine solids produced in the system, which are insufficiently removed from the water with the currently available techniques (Losordo et al., 1999; Chen et al. 1996; Chen et al. 1997). A high concentration of suspended solids has a negative influence on nitrification, water quality (Eding et al., 2006) and fish growth (Davidson et al., 2009). The problem can be reduced by adjusting the source of the nutrients, i.e. the feeds and the feeding strategies, the design of the tanks and their hydraulic characteristics, and the efficiency of the solids removal systems. Research priorities include:

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*Avoid feed spillage.* This requires studies on feed intake regulation and on feeding strategies for RAS.

*Increase feed efficiency.* This relates to more classic nutrition studies. The potential gain is less apparent, but, especially because of the significant changes to be expected in the used resources, it remains very important to take the digestibility and utilization of feed ingredients into account when developing specific RAS diets.

*Optimization of the consistency, water stability and composition of the faeces.* The targeted outcome of this line of studies is to produce faeces which can be easily removed from the water, produce less fine solids, and when produced can be easily fermented by the microbial community in the system. Recent studies start to shed some light into these interactions. Amirkolaie et al. (2006) showed that a higher inclusion of starch in the diet of Nile tilapia resulted in a higher viscosity of the digesta which contributed to higher faeces removal efficiency in the RAS. These authors also showed that the degree of gelatinization in the diet affects faeces removal rate. In another study, Amrikolaie et al. (2005) showed that the inclusion of insoluble non-starch polysaccharide (cellulose) in the diet also improves the removal efficiency of particles in RAS by increasing faeces recovery. Brinker (2007) also showed that supplementing rainbow trout feed with high-viscosity guar gum resulted in improved faecal stability and an increase of the mechanical treatment efficiency (Brinker et al., 2005). The above studies call for more

*Technology development and implementation for (fine) solids removal.* Most freshwater systems use drum filters or similar devices to filter larger solids particles from the tank effluents and rely on subsequent fixed bed bio-filters to remove the fine solids (Losordo et al., 1999). In marine RAS, drum filters or equivalent devices are often combined with foam fractionation systems (protein skimmers) to improve the fine suspended solids removal. Until today, there is no unambiguous and clear answer how to control and remove the different solids fractions in a cost effective and treatment efficient way. Further, the hydraulic characteristics within the rearing tank and the solids removal system affect the efficiency of solids removal (Klapisis and Burley, 1984; Losordo et al., 1999). Technology innovations in this area should take tank design, solids removal system and the hydraulic conditions into account. Finally, in marine RAS, ozone is often used to improve the water quality in the system (Suantika et al., 2001; Tango and Gagnon, 2003; Wolters et al., 2009). Ozone may alter the characteristics of the fine solids (Tango and Gagnon, 2003), thereby improving the effectiveness of the foam fractionators in the system. However, the interaction still needs further research.

## Nitrogen

In most RAS systems, nitrogen is removed by a combination of moving bed and fixed bed nitrification reactors and, in some cases, additional denitrification reactors (Losordo et al., 1999). The *nitrification* process in RAS is hampered by the level of organic matter entering the bio-filters (Eding et al., 2006). As a result, both autotrophic and heterotrophic bacteria are growing in the reactors. The challenge is to enable nitrification reactors to work as chemo-autotrophic as possible, e.g., by minimizing the organic carbon (OC) in the influent of the nitrification reactor. Therefore, major objectives for research are: Separate the OC and TAN removal in different treatment steps;

Make a mixed reactor, in which OC and TAN removal are combined. In such a reactor, the first part of the reactor will focus on OC removal, the second part on TAN removal;

To continue research on constructed wetland technologies (e.g. PROPReproject)

In contrast, a *denitrification* reactor in RAS requires an influent with a high C: N ratio (van Rijn, 2006). Often external carbon sources are used, such as methanol, ethanol or glucose (Sauthier et al., 1998). Ongoing research explores possibilities to use internal carbon sources (e.g., the solid waste produced by the fish, Klas et al., 2006). This is a spectacular development because it provides the theoretical perspective to close a RAS to nearly 100% from an ecological point of view. Furthermore, the incorporation of a denitrification reactor in freshwater RAS has been predicted to reduce cost price by 10% despite the higher investment and operating costs (Eding et al., 2009). However, the technology is still immature and the cost effectiveness needs to be better understood.

New purification technologies, such as the **anaerobic ammonium-oxidizing (Anammox)** technology, which converts TAN directly into nitrogen gas (e.g. Gut et al., 2006, van Rijn et al., 2006), deserve to be fully tested and their feasibility for RAS needs to be investigated. The limited number of studies using this purifying technology in RAS (Tal et al., 2006, 2009) shows promising results. Tal et al. (2009) using **Anammox** achieved 99% water recycling in a marine RAS.

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Worth noting is also the recent development of granular sludge systems (Yilmaz et al., 2008; Di Iaconi et al., 2010) that could be particularly interesting in combining simultaneously nitrification, denitrification and P-removal in one single system.

In addition, the microbial ecology of the nitrification/denitrification reactor systems in RAS deserves also further study. It is believed that fundamental research in this area may provide innovations which may alter and/or improve the reactor performance in RAS drastically. Until today, the microbial community in reactors is difficult to control (Leonard et al., 2000, 2002; Michaud et al., 2006, 2009; **Schreier** et al., 2010) and many of the inefficiencies of the system originate from this.

Research priorities to improve the denitrification process in RAS include:

- Design systems in which nutrient inputs (feeds) optimize concurrently fish growth and welfare, and water purification (waste removal and- nitrification/denitrification performance).
- Develop denitrification systems using the internal RAS sludge as carbon source
- Explore the possibility to steer microbial communities in RAS

#### Phosphate

Partly as a result of prevailing water management and legislation in most EU member states, most current RAS do not focus on specific phosphate removal systems, leading to accumulation of PO<sub>4</sub> in the system water and relative high P levels in the RAS effluents (e.g. Martins et al., 2009a). The efficiency and cost effectiveness of phosphate removal is one of the most important barriers. Controlling phosphate levels is possible through one or a combination of the following methods:

Optimizing P-retention in the fish

*Fast removal of solids from the water* (to avoid leaching of phosphorus from the organic matrix)

*Dephosphatation techniques.* At this moment, only classic chemical flocculation (dephosphatation) is well established in freshwater RAS (e.g. Kamstra et al., 2001).

*Integrated multi-trophic aquaculture, IMTA* (end-of-the-pipe treatment by recycling phosphorus in other commodities, (e.g. Metaxa et al., 2006; Muangkeow et al., 2007).

Because of the expected future shortage in world phosphate resources, recycling and saving phosphorus should be a top research priority. When RAS are integrated in an integrated agriculture-aquaculture system (for example, with greenhouse cultures, e.g. <http://www.ecofutura.nl/theproject.htm>, <http://www.vigourfishion.nl/index.php/>, <http://attra.ncat.org/attra-pub/PDF/aquaponic.pdf>, Savidov et al., 2007), feeds should be adjusted in such a way, that all waste exported to the greenhouse plants, is easily mineralized and assimilated by the plants. This calls for feed formulations using nutrient digestibility and utilization data in fish together with nutrient assimilation data from the target plants.

#### Conclusions

‘Producing more food from the same area of land while reducing the environmental impacts requires what has been called *sustainable intensification*’ wrote Godfray et al. (2010) in a recent review about the challenge of feeding 9 billion people. The key question is how can more food (in the scope of this review, more fish) be produced sustainably? Considering all aquaculture production systems in use today, RAS offers the possibility to achieve a high production, maintaining optimal environmental conditions, securing animal welfare, while creating a minimum ecological impact. At present, the use of RAS is growing in Europe, for grow-out of freshwater (eel and catfish) and marine species (turbot, seabass and sole) but also for fingerling production of both freshwater and marine species. Recent research aiming to improve water treatment efficiency (denitrification reactors, sludge thickening technologies and ozone) allows reducing water refreshment rates, creating nearly closed systems, producing a small quantity of an easy to treat and valuable waste product for use in IAA or IMTA systems. Despite the recent developments that will certainly foster the environmental sustainability of RAS, the potential accumulation of substances in the water as a consequence of reduced water refreshment rates may pose new challenges. A deeper understanding of the interaction between the fish and the system will help facing these challenges.

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## Acknowledgements

C.I.M. Martins was supported by a grant provided by the Foundation for Science and Technology, Portugal (SFRH/BPD/42015/2007). Further financial support came from the Dutch Ministry of Agriculture, Nature Conservation and Food Quality (LNV bestek Duurzame viskweek Ond/2005/08/01) and the SUSTAINAQUA project (co-funded by the European Commission; for more details on the project and its twenty-three partners visit [www.sustainaqua.org](http://www.sustainaqua.org)).

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(Continued on next page)

**Table 1.** Grow-out production (metric tones/year) in RAS from 1986 until 2009. Data were obtained by interviews with relevant stakeholders (feed industry, farmers, associations etc) in the different European countries.

[illegible]



**Table 2.** Comparison of fingerling production (fingerling heads/year) in RAS between 2005 and 2009. Data were obtained by interviews with relevant stakeholders (feed industry, farmers, associations etc) in the different European countries.

|                        | 2005     | 2009     |
|------------------------|----------|----------|
| Bosnia and Herzegovina |          | 260000   |
| Bulgaria               |          | 5000000  |
| Czech Republic         |          | 60000000 |
| Faroe Islands          | 4000000  | 6500000  |
| France                 | 61400000 | 73729000 |
| Hungary                | 650000   | 367500   |
| Italy                  | 90000000 |          |
| Norway                 | 350000   | 3800000  |
| Portugal               | 10000000 |          |
| Shetlands              | 500000   |          |
| Spain                  | 5000000  |          |
| United Kingdom         | 2500000  | 1550000  |

**Table 3. Energy consumption by various fish production systems (fisheries and aquaculture).**

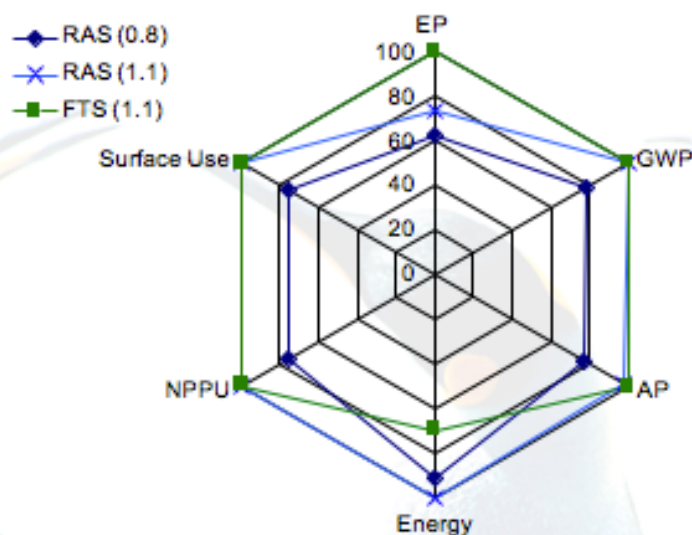
| Species            |  | Production                    | Feed                    | Functioning                | Total                   |
|--------------------|--|-------------------------------|-------------------------|----------------------------|-------------------------|
|                    |  | tool* (kWh*kg <sup>-1</sup> ) | (kWh*kg <sup>-1</sup> ) | ** (kWh*kg <sup>-1</sup> ) | (kWh*kg <sup>-1</sup> ) |
|                    |  | 1 <sub>1</sub>                |                         |                            |                         |
| <b>Fisheries</b>   | <b>Herring (1)</b>                     | <b>0.25</b>                   | <b>0</b>                | <b>1</b>                   | <b>1.25</b>             |
|                    | <b>Cod (1)</b>                         | <b>1-5</b>                    | <b>0</b>                | <b>4-16</b>                | <b>5-21</b>             |
|                    | <b>Lobster (1)</b>                     | <b>10-22</b>                  | <b>0</b>                | <b>40-90</b>               | <b>50-112</b>           |
| <b>Aquaculture</b> | <b>Mussel (2)</b>                      |                               | <b>0</b>                |                            | <b>0.7</b>              |
|                    | <b>Trout, FTS (3,4)</b>                | <b>3</b>                      | <b>5-6</b>              | <b>1-2</b>                 | <b>10-12</b>            |
|                    | <b>Trout or bass RAS (4)</b>           | <b>6-7</b>                    | <b>5-6</b>              | <b>3-6</b>                 | <b>15-20</b>            |
|                    | <b>Large trout FTS (4,5)</b>           |                               |                         |                            | <b>22</b>               |
|                    | <b>Oyster (6)</b>                      |                               |                         |                            | <b>26</b>               |
|                    | <b>Tilapia conventional RAS (7)</b>    |                               |                         | <b>5.2</b>                 |                         |
|                    | <b>Tilapia denitrification RAS (7)</b> |                               |                         | <b>2.2</b>                 |                         |

1) Ziegler et al., 2006, (2) Thrane, 2006, (3) Papatryphon et al., 2004a,b (4) Roque d'Orbcastel et al., 2009c, (5) Aubin et al., 2009, (6) Pimentel et al, 1996, (7) Eding et al., 2009; \* means energy to build the system; \*\* means energy needed to operate the system

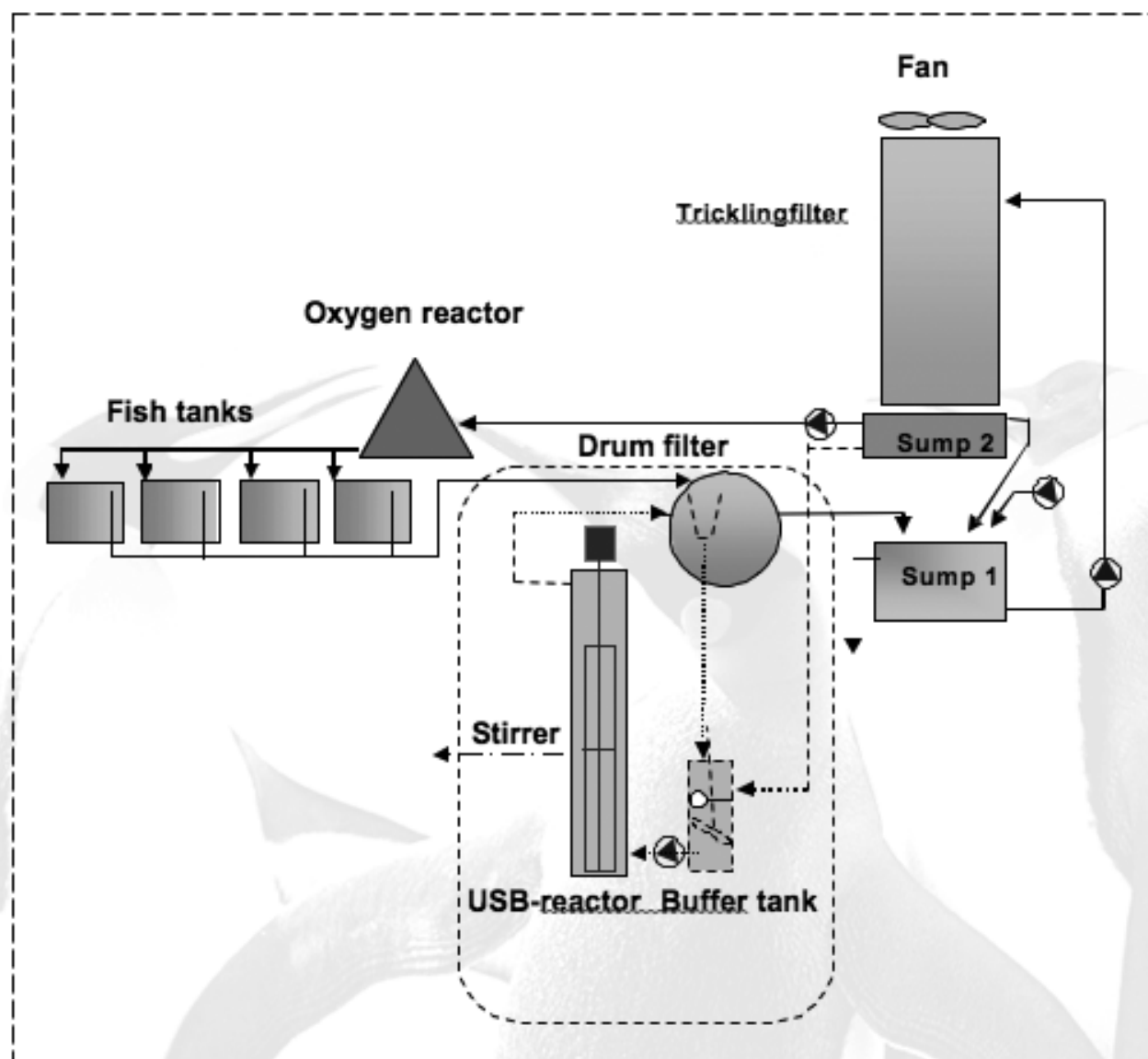
**Table 4.** Comparison of environmental sustainability indicators for a hypothetical 100 MT/y intensive tilapia farm with conventional RAS and RAS using a denitrification reactor (Eding et al., 2009).

|                                  | <i>Conventional RAS</i> | <i>Denitrification RAS</i> |
|----------------------------------|-------------------------|----------------------------|
| <b>Resource use</b>              |                         |                            |
| Fingerlings (#/kg)               | 1.2                     | 1.2                        |
| Feed (kg/kg)                     | 1.22                    | 1.22                       |
| Electricity (kWh/kg)             | 1.8                     | 2.2                        |
| Heating (kWh/kg)                 | 3.4                     | 0.0                        |
| Water (L/kg)                     | 238                     | 38                         |
| Oxygen (kg/kg)                   | 1.18                    | 1.26                       |
| Bicarbonate (g/kg)               | 252                     | 107 <sup>a</sup>           |
| Labour (h/MT)                    | 12.5                    | 13.1                       |
| <b>Nutrient utilization</b>      |                         |                            |
| Nitrogen (% of input)            | 32                      | 32                         |
| Phosphorus (% of input)          | 43                      | 43                         |
| COD (% of input)                 | 32                      | 32                         |
| TOD (% of input)                 | 32                      | 32                         |
| <b>Waste discharge</b>           |                         |                            |
| <b>Nitrogen</b>                  |                         |                            |
| Solid (g/kg)                     | 8.5                     | 2.6                        |
| Dissolved (g/kg)                 | 37.4                    | 5.9                        |
| <b>Phosphorus</b>                |                         |                            |
| Solid (g/kg)                     | 4.5                     | 7.2                        |
| Dissolved (g/kg)                 | 3.8                     | 1.3                        |
| <b>COD</b>                       |                         |                            |
| Solid (g/kg)                     | 189                     | 84                         |
| Dissolved (g/kg)                 | 40                      | 9                          |
| <b>TOD</b>                       |                         |                            |
| Solid (g/kg)                     | 227                     | 95                         |
| Dissolved (g/kg)                 | 48                      | 11                         |
| CO <sub>2</sub> (kg/kg incl gas) | 1.58                    | 1.10                       |
| TDS (g/kg)                       | 62                      | 28                         |
| Conductivity (μS/cm)             | 1060                    | 2000                       |

## Figures



**Figure 1.** Comparison of the environmental impact of three scenarios of trout production systems (average production of 500 tons per year): 1) traditional flow through farm (FTS), 2) hypothetical farm in RAS (with FCR of 1.1) and 3) RAS (with FCR of 0.8 ). RAS data were extrapolated from 2 years of experimental data obtained on pilot Danish model farms (Roque d'Orbcastel, 2008); environmental impacts are represented in proportion of the largest impact (%).



**Figure 2.** Innovative RAS using a denitrification (USB) reactor. Water flows from rearing tanks– drum filter – sump 1– trickling filter– sump 2–rearing tanks. One parallel flow across the denitrification reactor, using only fecal carbon as energy source, flows from the drum filter – buffer tank– denitrifying reactor– drum filter.

## **AGRICULTURAL SYSTEMS, POVERTY ERADICATION AND FOOD SECURITY -THE CASE OF COOPERATIVE BUSINESS MODEL IN BOTSWANA**

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The cooperative business model could be adopted to drive selected agricultural systems for Eradication of food poverty, in particular, and that generally, the calculated combination of agricultural systems and cooperatives, could become a mainstay in the in the eradication of poverty in all its forms.

There is limited agricultural production space and such scarcity of irrigation water in arid and semi arid parts of world. A more effective land and water use system urgently required. There is lack of common value and common purpose in the membership of our clusters and cooperatives. Also, clusters and cooperatives are formed by members who have not demonstrated any skill nor have they demonstrated readiness to work. In this way our clusters and cooperatives are self destructive

The space at households can adequately accommodate container gardens in urban and rural setting, for eradication of hunger and malnutrition, with use of minimal fraction of household portable water. The eradication of household food insecurity becomes a precursor for national Poverty reduction and food security. The technically inclined cooperative membership (individual and cluster champions) and the common value and common purpose oriented drive of the cooperatives are likely to have a higher level of productivity and success in business development and management

Target group includes;

- Community Development Trusts and Cooperatives
- Civil society based organizations,(including; religious organizations and social or sporting clubs)
- Disciplined forces (including; Soldiers, Police officers and Prison warders)

Training of group of civil institution based individuals in the establishment and management of various levels of household container gardens, followed by establishment of Commodity clusters and cooperatives where adequate space is accompanied by reliable water source. Number of people trained and graduating from hunger and malnutrition, and number of clusters and cooperatives, effectively contributing to Poverty Reduction, food security and wealth creation.



## RESTAURING THE MARINE RESERVE EL PELADO (ECUADOR) WITH *Spondylus limbatus*: STOCKING SUCCESS OF HATCHERY PRODUCED JUVENILES

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The Spondylids played an important cultural, political and religious role in Mesoamerican and Andean cultures. Today its symbolism remains in Latin American societies such as Mexico, Peru and particularly Ecuador. Excessive and uncontrolled exploitation of natural stocks in Ecuador has conditioned the resource near to extinction. Since 2009, the fishery of *Spondylus* was permanently banned in the Ecuadorian Coast. However, recovery of natural populations has not been evidenced, in part probably due to continuous illegal extraction. In an attempt to contribute with the conservation of this valuable biological resource, a series of research-oriented studies of *Spondylus* aquaculture and stock enhancement activities has been carried out at CENAIM-ESPOL with support of the Undersecretary of Aquaculture of the Ministry of Agriculture, Livestock, Aquaculture and Fisheries (MAGAP) and the Technical Secretariat for Higher Education, Science, Technology and Innovation of Ecuador (SENESCYT).

This research focused mainly on husbandry hatchery techniques for the reproduction and production of *Spondylus limbatus* spats. Our findings revealed particular post-metamorphic physiological features unique to *S. limbatus*, important for the settlement and cementation of competent post-larvae on hard substrates (Fig. 1). Over 5 thousand *S. limbatus* juveniles were obtained in the laboratory of which about 400 individuals were stocked in selected sites of the marine reserve El Pelado off the coast of Ecuador between 2015 and 2016. Monitoring of introduced juveniles were performed on a monthly basis to register growth and survival (Fig. 2).

The establishment of this Spondylid back into its natural environment may create a repopulation nuclei promoting a greater and more effective dissemination of its kind, fostering larvae settlement in neighboring zones, leading to new recruitments of *S. limbatus* in this part of the eastern pacific coast for the future.

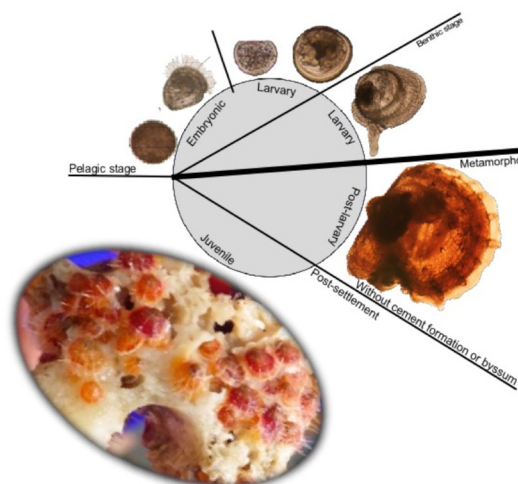


Figure 1. Early life cycle of *S. limbatus*

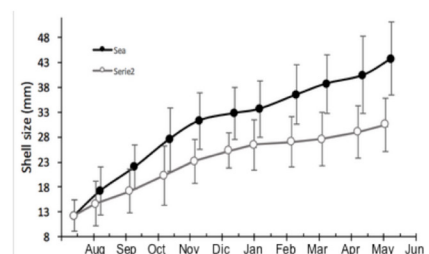


Figure 2. Growth and survival of *S. limbatus*



## **THE 3 PILLARS OF UNIVERSITY COOPERATION: EDUCATION, RESEARCH AND SERVICE TO SOCIETY**

Patrick Sorgeloos

Prof. em. Ghent University, Belgium  
Past-President of the World Aquaculture Society  
Chairman ASEM Aquaculture Platform and EATIP Working Group “International Cooperation”

As the aquaculture industry is facing big challenges an important engagement is expected from the universities to help realizing the full potential of a sustainable blue economy.

Traditionally universities have been focusing on education and eventually on research as well. It is however, only in recent years that the importance of the 3<sup>rd</sup> pillar “service to society” became properly acknowledged.

Universities should adopt the latest educational tools to guarantee best capacity building and life-long learning practices for young graduates and established farmers respectively. Interuniversity cooperation at the national and international level offers opportunities for more efficient training programs through credit exchange and double degree programs.

In comparison with the production of terrestrial plants and animals we are far behind in our knowledge of different aspects of the farming of aquaculture species. We need a more concerted, interdisciplinary and knowledge-based approach in aquaculture research in close interaction with research institutes, industry and society at large.

Universities could also take more responsibility in society and engage in a pro-active way in outreach and interaction with all stakeholders, e.g. farmers, service industries, knowledge centers, consumers and policy makers.

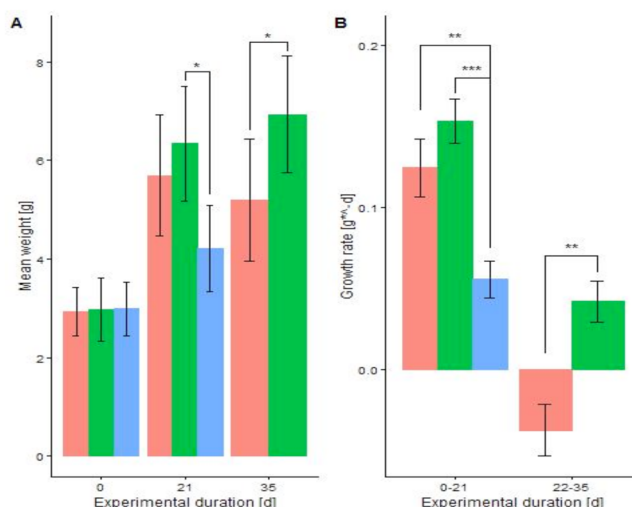
## POLYCULTURE OF SANDFISH *Holothuria scabra* AND PACIFIC WHITE SHRIMP *Litopenaeus vannamei* – A VIABLE APPROACH FOR A SUSTAINABLE FUTURE?

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Aquaculture monocultures can result in environmental degradation through excessive waste loading. In response to this, interest in polycultures and integrated multi-trophic aquaculture (IMTA) has increased in recent decades, with many species combinations including sea cucumbers suitable to reduce the amount of waste and nutrients in the water column and counteract environmental degradation. However, the feasibility of sea cucumber-shrimp co-cultures has not yet been confirmed. In the current study the co-culture of medium sized Sandfish, *Holothuria scabra* (1–8 g) and juvenile Pacific white shrimp, *Litopenaeus vannamei* is shown to be fundamentally feasible in controlled feeding trials.

Highest sandfish survival rates over the first three weeks were observed in co-culture with shrimp as compared to *H. scabra* monocultures. During this time, sandfish growth rates were, however, significantly higher in monocultures, with highest growth rates of  $0.15 \text{ g} \cdot \text{d}^{-1}$  in monoculture receiving a mixture of Algamac and shrimp diet and lowest growth rates of  $0.06 \text{ g} \cdot \text{d}^{-1}$  in the co-culture approach (Fig. 1). Sediment C/N values were significantly lower in co-culture compared to monocultures over the experimental duration with the exception of week five. Despite strong growth, all sandfish in the co-culture died within one week of the first sediment exchange (after 21 experimental days). Thus, making it impossible to compare growth rates of sandfish over the entire experimental duration. Results demonstrate that medium sized sandfish and juvenile shrimps co-culture can be possible, however apparent shrimp-sea cucumber interactions lead to sea cucumber deaths after a period of 3-4 week. Hence future development requires indirect co-culture methods co-culture towards to exclude negative interaction. The lower growth rates in the co-culture compared to the monocultures may be an indicator for insufficient food supply for sandfish in the co-culture. These results can be seen as a further step to gain knowledge for viable sandfish–shrimp co-culture. However, so far the knowledge is limited to specific stages of both, sandfish and shrimp, and has to be tested throughout the shrimp production cycle as a minimum.



**Figure 1: Mean weight [g] (A) and mean growth rate [ $\text{g} \cdot \text{d}^{-1}$ ] (B) of *H. scabra* over the experimental duration.** Red bars represent treatment 1 (*H. scabra* monoculture only Algamac), green treatment 2 (*H. scabra* monoculture, Algamac and shrimp pellets) and blue treatment 3 (polyculture). Error bars indicate standard error. Stars indicate the significance:  $p < 0.0001 = ***$ ,  $p < 0.001 = **$ ,  $p < 0.05 = *$

## **ARTEMIA SURVIVES IN LAKE BUNYAMPAKA SALINE WATER, AN INLAND SALINE LAKE IN UGANDA**

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Ugandan aquaculture is in a process of development however access to an affordable live food, i.e. *Artemia*, source is still a bottleneck. This is why a feasibility of domestic *Artemia* production was undertaken. Uganda being a landlocked country, the only opportunity lies in the salt lakes in the west of the country and saline water from one of these lakes was used in this study. Two *Artemia* strains, Great Salt Lake (GSL) a dominant strain on the market and Vinh Chau (VC) a strain that is by far the most inoculated strain in the world, were assayed for their responses in Lake Bunyampaka water. This study was carried out under laboratory conditions ( $28 \pm 1^\circ\text{C}$ ; pH 9; constant aeration and light) where salinity effects upon special characteristics of the two *Artemia* strains, such as survival, growth and reproductive traits were recorded. The organisms were fed with live freshly cultured *Tetraselmis suecica* ad libitum. This evaluation revealed that the two strains examined were able to survive, grow and even reproduce in diluted Lake Bunyampaka saline water. The VC strain performed better than the GSL at both salinities. Vinh Chau strain seems to be a more effective coloniser of Lake Bunyampaka saline water. The data presented in this study may generate useful suggestions for proper inoculation of *Artemia* in Lake Bunyampaka saline lake in western Uganda although further experimentation is needed.

## **EVALUATION OF GENETICALLY MODIFIED SALMON IN A COMPARATIVE GROWTH TRIAL WITH NON-GENETICALLY MODIFIED ATLANTIC SALMON *Salmo salar* AT AN APPROVED GM RESEARCH FACILITY IN SOUTH AFRICA**

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Genetically modified fish (GM fish) are organisms from the taxonomic clade which includes the classes Agnatha (jawless fish), Chondrichthyes (cartilaginous fish) and Osteichthyes (bony fish) whose genetic material (DNA) has been altered using genetic engineering techniques. In most cases, the aim is to introduce a new trait to the fish which does not occur naturally in the species, i.e. transgenesis. GM fish are already used in scientific research and kept as ornamental fish for pets. They are being developed as environmental pollutant sentinels and for use in aquaculture food production. In 2015, the AquAdvantage® salmon was approved by the US Food and Drug Administration (FDA) for commercial production, sale and consumption, making it the first genetically modified animal to be approved for human consumption.

AquAdvantage® salmon is a genetically modified (GM) Atlantic salmon developed by AquaBounty Technologies. A growth hormone-regulating gene from a Pacific Chinook salmon and a promoter from an ocean pout were added to the Atlantic's 40,000 genes. These genes enable it to grow year-round instead of only during spring and summer. The purpose of the modification is to increase the speed at which the fish grows without affecting its ultimate size or other qualities. The fish grows to market size in 16 to 18 months rather than three years. The latter figure refers to varieties whose growth rate has already been improved by 2:1 as a result of traditional selective breeding.

Conventional salmon growers publicly challenged the claimed growth rates. It is important that comparative growth trials are done between the GM salmon and non-GM salmon at a local independent research institution within a registered and approved physically contained GM research and development facility for South Africa. This research trial can be concluded within 1 year and will be performed on Welgevallen Experimental Farm in Stellenbosch. Additionally, risk analyses of GM aquatic organisms will have to be conducted as for any other GMOs and include three activities, i.e. risk assessment, risk management and risk communication.

## **DEVELOPMENT OF A MODULAR SCALABLE, INTEGRATED FARMING AND ECONOMIC FEASIBLE AQUAPONICS SYSTEM**

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Global aquaculture has been expanding at a rate of approximately 9% per year for more than 20 years now, and it is projected to continue growing at a very rapid rate into the foreseeable future. Aquaculture engineering, innovation and new technology will play an essential role in global aquaculture development. These subjects are key in production, output and efficiencies and would require a lot more focus and attention in the future. The increased focus on the interaction between the aquaculture industry and society will be an imminent cast-iron certainty.

Given all the advantages of Aquaponics as a technology for food production systems, economic feasibility and scale of production still require more research and development attention. A new modular and fully integrated Aquaponics system was designed and proof of concept for economic feasibility will take place during the first two years of operation.

Commercialization of this fully integrated and modular system, would simple be a role out and replication of the original turnkey unit once all the efficiencies were optimized during the developmental phase.

## MARICULTURE OF KELPS IN SOUTHEAST ALASKA

Michael S. Stekoll\*, Tamsen Peebles, Ann Thomson

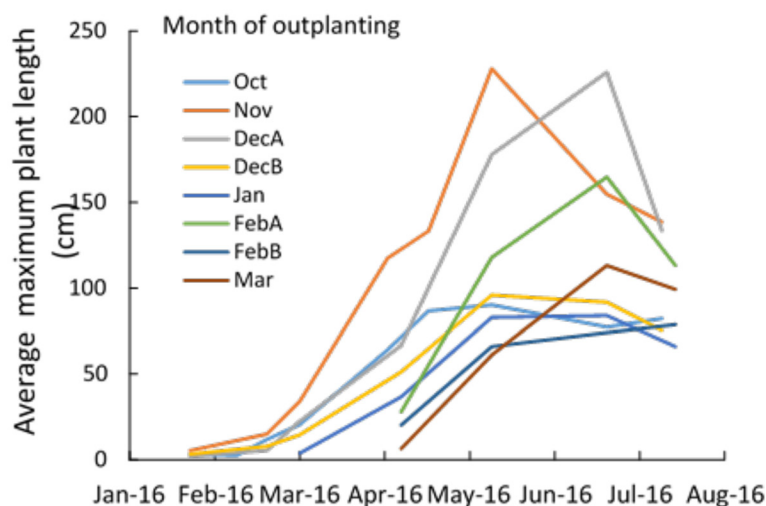
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Although mariculture operations in Alaska have existed for over 100 years, no successful effort has been made to create a viable commercial seaweed venture in the state. Due to recent interest in growing seaweeds as a source of nutrition, we have initiated research on the culture of several species of seaweeds that have commercial potential. In addition, in cooperation with private industry, we are working with state agencies to create a pathway for the permitting of commercial seaweed hatcheries and farms.

Current research is focusing on the mariculture of kelps. “Cremona” strings seeded with *Saccharina latissimi*, *Alaria marginata* and *Nereocystis luetkeana* have been placed on longlines and outplanted in the nearshore in Juneau and Sitka, Alaska. Growth of these kelps was measured as a function of time, depth and the location of the outplantings. Substantial growth occurs throughout the winter and spring with daily growth rates as high as 5 percent. Outplants in November grew over two meters in length by April. All outplantings ceased net growth and deteriorated during late spring and early summer. It is likely that light is limiting for growth in the winter and nutrients are limiting for growth in the late spring and summer. Outplantings in Sitka grew larger than those in Juneau.

The university in conjunction with private industry has been permitted for a commercial kelp hatchery. This year several kilometers of seeded strings on pipes have been produced and shipped to the first commercial kelp farms in the state.

Ongoing research will continue to refine parameters for optimal production and quality of the commercially grown kelps.



Growth of *Saccharina* sp. on longlines near Juneau, AK. Each line represents the growth of *Saccharina* outplanted during the corresponding month

## TEMPORAL AND SPATIAL VARIABILITY IN CULTURED MUSSEL CONDITION (BODY DIMENSIONS, GONAD STATE, AND FATTY AND AMINO ACID PROFILES) IN RELATION TO ENVIRONMENTAL SIGNALS

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This study determined the relationship between the body condition of the mussel *Perna canaliculus* cultured in three difficult geographical locations in New Zealand and environmental variability over 2 years. The body parameters were shell dimension, shell weight and strength, body weight, gonad weight, reproductive state, and whole body fatty and amino acid profiles. The environmental variables were dissolved and particulate nitrogen, VSS, chlorophyll-a, phytoplankton carbon, and anomalies in SST, wind stress and river inputs.

Multiple regression models identified significant relationships between mussel condition and environmental variability to enable the construction of an interpolated index of mussel health. This tool can be used to inform farmers on harvesting strategies: Does the mussel go on the culinary or nutraceutical table?



## VULNERABILITY TO CLIMATE CHANGE: EXPOSURE, SENSITIVITY & ADAPTIVE CAPACITY OF MOLLUSCAN SHELLFISH AQUACULTURE

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Human-induced climate change is changing the physical and biological processes within the marine environment from local to global scales. The shellfish aquaculture industry is an important contributor to the economy of many coastal nations worldwide. Changes in environmental variables such as pH, temperature, primary productivity, and extreme weather events that are directly linked to climate change will have direct effects on aquaculture.

We compared the vulnerability of mollusc shellfish aquaculture industries to climate change using an indicator-based approach on a global scale. We used a vulnerability assessment (VA) that was composed of equally-weighted layers: exposure (E), sensitivity (S) and adaptive capacity (AC). Exposure to future changes in sea surface temperature, ocean acidification, extreme weather events, and primary productivity was modelled to the year 2100.

The level of exposure to climate change variables was dependent on latitude; high latitudes will experience the greatest increase in temperatures and lowered aragonite saturation, posing problems for calcifying organisms. In low latitudes, temperatures will rise to levels exceeding current habitat tolerances for many cultured species including molluscs. Low adaptive capacity was seen in developing and the least developed countries and those characterised by high political instability and/or low diversity in their aquaculture industry. Peru is predicted to have the greatest sensitivity to climate-change because of its reliance on molluscan culture as a source of protein and the relative importance of this industry to the national economy (Figure 1).

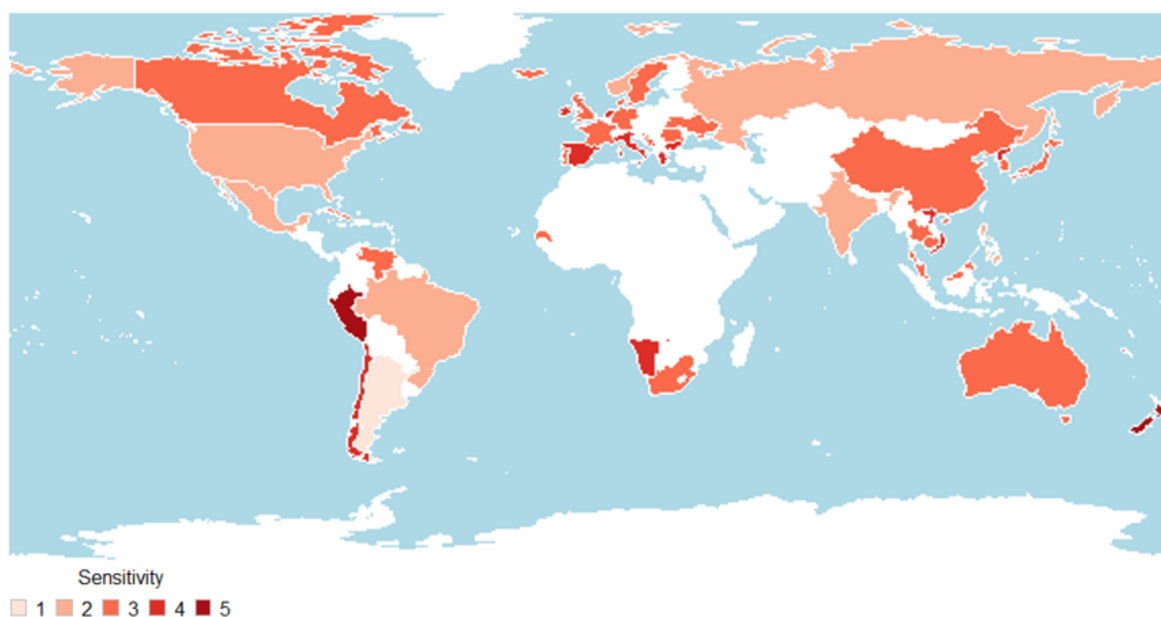


FIGURE 1: Shellfish aquaculture industry sensitivity to climate change. Missing or incomplete data shown in white.

## USE OF PERACETIC ACID TO DISINFECT WATER: TOXICITY TO FISH

David L. Straus\*, Thomas Meinelt, Dibo Liu and Lars-Flemming Pedersen

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There has been strong interest in aquaculture for the use of peracetic acid (PAA) as a disinfectant to prevent freshwater fish pathogens. PAA is a stabilized mixture of acetic acid, hydrogen peroxide and water that does not leave dangerous residues in the environment when it breaks down as most compounds do. It is a promising disinfectant in the US aquaculture industry to control parasites and fungus. The US Environmental Protection Agency (EPA) first registered PAA as an antimicrobial in 1985 for indoor use on hard surfaces (hospitals). EPA registrations now include: sanitation in food/beverage plants, agricultural premises, wineries/breweries, greenhouse equipment, and animal housing; it is also used to prevent bio-film formation in paper/pulp industries and as a disinfectant for wastewater treatment. PAA is used in Europe, and our international collaborations have studied its effectiveness to many pathogens. However, there is a lack of data on its toxicity to fish.

Twelve fish species (Table 1) were exposed to PAA in well water to determine its toxicity. Experiments were designed to provide the 24 h LC50 (median lethal concentration), LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration) values for each species at ~23°C. Ten fish were placed in aquaria containing 10 L of well water (pH = 7.5, alkalinity = 200 mg/L, hardness = 125 mg/L). Each experiment consisted of 6 PAA concentrations and an untreated control; there were 3 replicates of each aquaria. The mean LC50 value for all species tested was 5.3 mg/L PAA with the range of 2.8 mg/L to 9.3 mg/L. Black fathead minnows and blue tilapia were most and least sensitive, respectively. The mean NOEC value for all species tested was 3.7 mg/L PAA with the range of 1.9 mg/L to 5.8 mg/L. This information can be used to approximate safe treatment levels; however, application must be tailored to fit specific species and individual water quality and chemistry.

Table 1. List of species used in study.

| Latin Name                               | Common Name          |
|--|----------------------|
| <i>Pimephales promelas</i>               | Black Fathead Minnow |
| <i>Notemigonus crysoleucas</i>           | Golden Shiner        |
| <i>Oncorhynchus mykiss</i>               | Rainbow Trout        |
| <i>Ctenopharyngodon idella</i>           | Grass Carp           |
| <i>Carassius auratus</i>                 | Goldfish             |
| <i>Lepomis macrochirus</i>               | Blue Gill            |
| <i>Ictalurus punctatus</i>               | Channel Catfish      |
| <i>Micropterus salmoides</i>             | Large Mouth Bass     |
| <i>Pomoxis nigromaculatus</i>            | Black Nose Crappie   |
| <i>Sander vitreus</i>                    | Walleye              |
| <i>M. chrysops</i> x <i>M. saxatilis</i> | Hybrid Striped Bass  |
| <i>Oreochromis aureus</i>                | Blue Tilapia         |

## **SOME COMMON THEMES FOR USAID FARMER-TO-FARMER SMALL SCALE AQUACULTURE ASSISTANCE IN SUB-SAHARAN AFRICA: MAINTAINING POST-HARVEST FISH QUALITY**

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The food quality of fish only deteriorates after harvest; the only question is how fast which depends upon how it is handled and local environmental conditions. Many fish markets in Sub-Saharan Africa see recently harvested or captured fish lose their value for human food by the end of the day and are either discarded or further processed in a less than satisfactory way for sale again the next day. Small improvements on traditional drying and smoking methods can help this. Some make smoking or drying cheaper and practical even in the wet season, and, if there are local fabricators in the area, more sophisticated but still relatively simple smokers greatly increase both the quantity of smoked product but also the variety available to the end consumers. Fisher/fish farmer cooperatives can pursue ice, refrigeration, freezing, live sale and canning that individuals often cannot afford. Consistent availability of electricity is a constraining factor for many areas, but even when it is available, training in refrigeration/freezing equipment repair can make a big difference. Low tech live sale methods that are practiced in Asian developing nations can be used in developing African nations as well. Generally speaking, a cooperative greatly assists the practicality of canning to obtain even inexpensive equipment and supplies. Properly canned fish are shelf stable forever and make fish available for more than just the immediate time after harvest or fishing seasons. Cooperatives also facilitate, if not require, pre-harvest, pre-processing and pre-marketing planning because everyone wants to maximize their own profit share. Live fish are the highest quality, capturing the highest price, and this can often become possible because cooperatives allow structuring the value chain, where everyone has a job to do from harvest to market.

This training is one of the common themes private farmers request from the US Agency for International Development. USAID funds a volunteer program in developing countries called The John Ogonowski and Doug Bereuter Farmer-to-Farmer (F2F) program and contracts with NGOs to carry out aquaculture projects (among other agriculture projects) under this program in Africa. The NGO then requests volunteers from its network of aquaculture experts. Travel is usually covered by the agency; the client farmers have to share some of the local costs for the volunteer. This “bringing something to the table” ensures that training is not just for its own sake, but is seen by the host farmers as something very desirable and valuable to them, the results of which they are committed to using. NGOs that have included post-harvest fish quality projects include Winrock International, ACDI/VOCA, Catholic Relief Services, and possibly others.

## **SOME COMMON THEMES FOR USAID FARMER-TO-FARMER SMALL SCALE AQUACULTURE ASSISTANCE IN SUB-SAHARAN AFRICA: CREATING FISH FEEDS FROM LOCALLY AVAILABLE INGREDIENTS AND FABRICATED EQUIPMENT**

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Small scale fish farmers cannot afford to import fish feed in those areas without local feed mills. Finding locally available ingredients that can be used and identifying them and their quality as ingredients can make aquaculture, particularly of catfish, practical and profitable. Using extruders to make floating pelleted feed increase is desirable but out of reach fiscally when these machines must be purchased and imported from distant countries. One solution is to have local fabricators make them or something else that will result in floating feed. Cooperatives have a better chance of making this happen than individual fish farmers. At least one Sub-Saharan country, Senegal, procures fish meal and sells it to fish farmers, but then requires them to obtain the other ingredients, after which they will mill the feed for them. Wastes from beer brewing, palm oil extraction, abattoir wastes and other resources may not have a current local use otherwise and may be available for creating fish feed.

The training on fish feed production is one of the common themes private farmers request from the US Agency for International Development. USAID funds a volunteer program in developing countries called The John Ogonowski and Doug Bereuter Farmer-to-Farmer (F2F) program and contracts with NGOs to carry out aquaculture projects (among other agriculture projects) under this program in Africa. The NGO then requests volunteers from its network of aquaculture experts. Travel is usually covered by the agency; the client farmers have to share some of the local costs for the volunteer. This “bringing something to the table” ensures that training is not just for its own sake, but is seen by the host farmers as something very desirable and valuable to them, the results of which they are committed to using. NGOs that have fielded local fish feed projects include Winrock International, ACDI/VOCA, and possibly others. Another USAID program, Feed-the-Future, executed through another NGO, CNFA, is pursuing fish meal from by-catch and by-product of new fish processing plants in Liberia. This creates more infrastructure to support aquaculture in this country and fits hand-in-glove with the Farmer-to-Farmer project to create fish feeds from locally available ingredients. Instead of targeting forage fish, which sometimes negatively impacts marine ecosystems, using fish processing wastes and by-catch is environmentally sustainable.

## **SOME COMMON THEMES FOR USAID FARMER-TO-FARMER SMALL SCALE AQUACULTURE ASSISTANCE IN SUB-SAHARAN AFRICA: ALL-MALE TILAPIA PRODUCTION**

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All-male tilapia production is desirable because males grow faster and larger on the same amount of feed than females. All-male production methods include hand-sexing, sex reversal using methyltestosterone, Nile-blue and other hybrids and the use of YY male brood stock. Each method is appropriate for different levels of resource availability. The poorest fish farmer can follow hand sexing methods as long as they can see well close up and practice with increasingly smaller fish. Use of methyltestosterone works if the farmer has more resources than the subsistence level farmer but the fish generally must be sold within the country, or at least cannot be sold in the EU due to GMO concerns. Hybrids may not be practical if suitable species are not locally available or if hybrids of those particular stocks do not produce all males. The use of YY males requires much larger capital resources. In all cases, provision must be made for maintaining broodstock without inbreeding in ponds separate from production ponds. Thus cooperatives to share roles (e.g. fingerling versus market fish production) become very important for all but the largest fish farmers.

This training is one of the common themes private farmers request from the US Agency for International Development. USAID funds a volunteer program in developing countries called The John Ogonowski and Doug Bereuter Farmer-to-Farmer (F2F) program and contracts with NGOs to carry out aquaculture projects (among other agriculture projects) under this program in Africa. The NGO then requests volunteers from its network of aquaculture experts. Travel is usually covered by the agency; the client farmers have to share some of the local costs for the volunteer. This “bringing something to the table” ensures that training is not just for its own sake, but is seen by the host farmers as something very desirable and valuable to them, the results of which they are committed to using. NGOs that have fielded all-male tilapia and genetics/inbreeding avoidance projects include Winrock International, ACIDI/VOCA, CNFA and possibly others.

## **SOME COMMON THEMES FOR USAID FARMER-TO-FARMER SMALL SCALE AQUACULTURE ASSISTANCE IN SUB-SAHARAN AFRICA: SHARP TOOTH CATFISH FINGERLING PRODUCTION**

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The sharp tooth catfish industry can be divided into fingerling producers and grow-out farmers. Catfish cannot reliably be expected to spawn in ponds and those that do face high losses of juveniles for various reasons. Even in areas where catfish fingerling producers exist, they often want to sell only to larger buyers, leaving the home backyard fish farmer at a loss for a supply of fingerlings. Cooperatives can remedy this situation, but require training in spawning techniques and rearing fry to pond-hardy fingerling sizes. One knowledgeable and capable coop member can supply other members with these fingerlings. Commercially available hormones to induce egg maturation are available for reasonable prices, but training also includes the extraction and use of pituitary glands from local fish to achieve this end by that method as well.

This training is one of the common themes private farmers request from the US Agency for International Development. USAID funds a volunteer program in developing countries called The John Ogonowski and Doug Bereuter Farmer-to-Farmer (F2F) program and contracts with NGOs to carry out aquaculture projects (among other agriculture projects) under this program in Africa. The NGO then requests volunteers from its network of aquaculture experts. Travel is usually covered by the agency; the client farmers have to share some of the local costs for the volunteer. This “bringing something to the table” ensures that training is not just for its own sake, but is seen by the host farmers as something very desirable and valuable to them, the results of which they are committed to using. NGOs that have executed catfish fingerling production projects include Winrock International, ACDI/VOCA and possibly others.

## DETERMINING THE BIOAVAILABILITY OF PHOSPHATE FEED ADDITIVES WITH AFRICAN CATFISH *Clarias gariepinus*

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African catfish (*Clarias gariepinus*) is a popular aquaculture species in Africa, which and was used to evaluate feed ingredients derived from bycatch monkfish (*Lophius vomerinus*) heads. The fish heads were enzymatically hydrolysed and the bones was used to extract (leach) the bone minerals with acid to create fish bone dicalcium phosphate (FB-DCP) as a phosphate feed additive. The bioavailability and bioactivity of the FB-DCP as a phosphate feed supplement is illustrated with a feeding trail with African catfish juveniles (1 g).

The FB-DCP was used to replace rock dicalcium phosphate (R-DCP), which is a common ingredient, and fish bones (FB) as a phosphate supplement. The feed formulation (38% protein) is designed to be isonitrogenous and isocaloric diets in **Table 1**.

Other ingredients in the diet includes constant amounts of soya meal, extruded maize, starch, fish oil, soybean oil, mineral and vitamin premix, and the cellulose which is used as an inert variable ingredient. The ingredients is mixed, extruded with a 5mm die and dried at 55° overnight.

The diets were fed to the juvenile catfish for 70 days, as 4-5% of the fish bodyweight per day, three times a day (8h00, 12h00 and 16h00), in a recirculating aquaculture system with tanks of a proximate volume of 70 L and a water temperature of 26-28 °C.

During this trail, the fish was sampled after 28, 49, and 70 days, where the fish is anesthetized with MS-222 to reduce stress and the weight and length of each fish was taken.

This feeding trail is an ongoing investigation, where it will only end after 10 weeks, where blood samples was taken to analyse innate immunity for each diet. Furthermore, whole body composition and mineral composition to compare the bone structure development for each treatment.

The preliminary results in **Table 2** show good growth rate (SGR) and a good feed conversation ratio (FCR) over all the diets. With the preliminary results there are insignificant ( $p>0.05$ ) differences, and that all treatments preform equally with the phosphate feed additive.

From the preliminary results, the conclusion can be made that all feed supplements preform equally well. Therefore, the FB-DCP ingredient is suitable in animal feeds illustrated by the *in vivo* feeding trail with the African catfish.

**Table 1** Some ingredients in the formulated diets for African catfish ( $\text{g kg}^{-1}$ )

| Ingredient | 1  | 2  | 3  | 4  |
|------------|----|----|----|----|
| FB-DCP     | -  | -  | 16 | 8  |
| R-DCP      | 20 | -  | -  | 10 |
| FB         | -  | 30 | -  | -  |

**Table 2** Production parameters feeding trail results

|             | 1     | 2     | 3     | 4     |
|-------------|-------|-------|-------|-------|
| $W_0$ (g)   | 0.96  | 1.04  | 0.96  | 0.95  |
| $W_f$ (g)   | 31.16 | 29.85 | 31.39 | 30.53 |
| SGR (%/day) | 4.99  | 4.83  | 4.98  | 4.96  |
| FCR         | 0.82  | 0.82  | 0.89  | 0.89  |
| CF          | 0.70  | 0.69  | 0.70  | 0.70  |



## MYXOZOAN DETECTION AND IDENTIFICATION BY MEANS OF SILVER-NITRATE IMPREGNATION

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Myxozoans are regarded as harmful pathogens in aquaculture systems and have an impact on fish welfare either directly or indirectly. Myxozoans have been the direct cause of devastating mortalities in the aquaculture industry. The accurate identification of myxozoans to species level generally requires a combination of morphometric data from fresh live specimens, histology and molecular methods. These methods require dedicate equipment and methods that are often difficult to achieve under field conditions or on site at remote aquaculture facilities and often myxozoans are diagnosed from fish and never properly identified to species level. This paper proposes the simple method of silver-impregnation, a modification of Klein's technique, to identify both myxozoans and trichodinids on the same skin or gill smears. The advantage of this method is that it provides sufficient morphological information for the identification of both myxosporean and trichodinids to species level and they can be identified from the same slides. Further more the advantages of this method allow for sampling in remote locations and for medium to long storage of appropriated stained and mounted reference material for diagnostic and taxonomic purposes. Silver-nitrate impregnation was used in this study to identify seven *Myxobolus* species where two are possibly new to science based on spore morphology. This method can be used by fish health professionals in the Aquaculture industry for rapid species level discrimination of these pathogens.

## ACASESTUDYOF“FARMERTOFARMER”EXTENSIONOFSMALL-SCALEAQUACULTURE FOR LIVELIHOOD IMPROVEMENT IN MYANMAR

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“Farmer to Farmer” extension is an effective approach to extend an appropriate level of small-scale aquaculture to local farmers for their livelihood in Myanmar. It is an autonomous extension system among farmers with no much dependence on government extension service. In this system, core farmers are developed, who are expected to be fish seed producers to distribute healthy fish seeds (fish fries and fingerlings) with essential information of aquaculture techniques to neighboring famers in the area.

Japan International Cooperation Agency (JICA) in cooperation with Department of Fisheries (DoF), Myanmar has been implementing the technical cooperation project titled “Project for Small-scale Aquaculture Extension for Promotion of Livelihood of Rural Communities in Central Dry Zone (SAEP in CDZ)” since 2014. The main objective of the project is to improve livelihood of rural communities through extending low-input/simple aquaculture techniques, such as small-pond aquaculture and paddy-cum fish culture to local farmers.

One of the key issues identified to expedite such local aquaculture is to secure local fish seed production and its distribution together with essential technical information to local farmers. For this, the project develops core farmers as a focal point of extension.

In the training program, the core farmers practice two basic seed production techniques, 1) intermediate fry nursery technique (level-1), and 2) natural breeding and fry nursery technique (level-2). The former usually uses Rohu (*Labeo rohita*) fries, which are produced by near-by government or private hatcheries, and transfer to farmers’ ponds. The farmers’ ponds are in advance prepared by liming and fertilization to propagate planktons for initial feeds of the fries. The fries are nursed in the ponds for 1 to 3 months and got sold to neighboring farmers. The account balance is shown in Table-1. The latter is practiced by Common carp (*Cyprinus carpio*). Selected breeders are mated in Hapa-net set in the pond. Floating water grasses are also put in the Hapa-net as a spawning bed. The breeders are stimulated by showering water. Spawning usually takes place within 1 to 2 days after matting, and fertilized eggs stick to the grasses. The eggs hatch out in a few days. The fries are nursed for 1 to 2 months and sold out.

Since a key point of extension is to secure the local seed production and its distribution to local needs together with essential technical information. The core farmers have started playing this role in Myanmar. It also promotes sustainable extension practices among farmers without much dependence on government extension services.

Table-1. Model of account balance of seed production (level-1) in 1 cycle for a 600 m<sup>2</sup> pond

|                  | Unit: Kyats |             |         |
|------------------|-------------|-------------|---------|
|                  | income      | expenditure | balance |
| sale             | 414,200     |             |         |
| fry              |             | 15,000      |         |
| feed             |             | 8,000       |         |
| lime, fertilizer |             | 10,000      |         |
| fuel             |             | 11,500      |         |
| pond repair      |             | 110,000     |         |
| total            | 414,200     | 154,500     | 259,700 |

## OPPORTUNITIES, CHALLENGES AND REALITIES IN AFRICAN COMMERCIAL 738 AQUACULTURE

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Rapid urbanization and a growing middle class leading African consumers to eat a more animal-protein based diet. With farmed fish being among the most affordable animal proteins, a significant commercial, environmental and social opportunity is present. However, operating in a new industry in challenging geographies is also fraught with risk. The co-founders of Yalelo fish farm will discuss their experience in Zambia as it relates to those opportunities, challenges and realities. The presentation will discuss issues such as estimating investment capital required, availability of capital, regulation, importance of distribution, developing technical capacity among staff and operating amid an undeveloped value-chain. Opportunities such as market dynamics, realistic production assumptions, environmental benefits and product competitiveness vs other animal proteins will also be discussed.

Yalelo Limited is Africa's largest fish farm. Since founding in 2011 and commencing production in 2013, Yalelo has installed 11,000 tonnes of production capacity and has annualized sales of 7,500 tonnes of tilapia. The fish is farmed in an off-shore floating cage installation comprised of 48 HDPE cages. The company sells fresh and frozen fish which is distributed through a network of 15 in-house stores and 30 franchises. The company supports a growing network of out-growers which supplement production.



## ASSESSING THE CURRENT STATUS OF DIAGNOSTIC CAPACITY AND DISEASES OF ECONOMIC CONCERN IN SOUTH AFRICA'S FARMED TILAPIA (*Oreochromis spp.*)

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With global food security becoming a growing concern, the world's eyes have swung to Africa, as not only a potential area of untapped resource, but also a potential region as yet untouched by many of the devastating diseases affecting the tilapia aquaculture industry worldwide. The newly developing South African tilapia industry holds great economic potential, but its success will depend on determining what infectious diseases exist, the availability of laboratories able to perform diagnostic assays that identify or confirm the cause of tilapia disease outbreaks, and a concerted effort of veterinarians to educate farmers on how to recognise diseases and what biosecurity principles will assist preventing, controlling disease outbreaks.

Preliminary results of efforts to assist tilapia farmers, and an ongoing study to determine the extent of economically important tilapia diseases in South African tilapia farms, using a two-stage surveillance approach involving general (passive) and targeted (active) surveillance of infectious bacterial, viral, fungal and parasitic diseases will be discussed.

Starting in 2016, presentations at Farmers Day gatherings and local organisation meetings were organized to introduce and inform tilapia farmers to basic disease identification, sampling technique in the event of an outbreak, important links between husbandry and disease, and best biosecurity practices to follow. Farms willing to participate in general or targeted surveillance are visited to determine history of any disease, evaluated for evidence of clinical disease, and assessed in terms of what biosecurity procedures that might prevent or control disease are currently in place. To determine, confirm and identify diseases that might be present, normal, moribund and fish showing clinical signs will be collected, necropsied and sampled for further diagnostic confirmation assays performed at diagnostic laboratories identified as able to perform diagnostic assays.

General surveillance to determine if, or what, diseases existed on 43 tilapia farms with production facilities over 100 000 litres in the three largest tilapia producing provinces of South Africa (Gauteng, North West, and Limpopo) was initiated in 2017. Active surveillance involving follow up epidemiologic investigation of disease outbreaks on these, and additional farms in other South African provinces, to determine the prevalence of diseases, their sources and reasons for an outbreak, and possible biosecurity procedures a farm might implement to control and eradicate the disease, and prevent future outbreaks, will continue through 2018.

Once completed in 2018, this work is hoped to provide important information on the prevalence of economically important tilapia diseases, determine South Africa's current diagnostic laboratory capabilities, and assist producers develop biosecurity programs that would help prevent, control and eradicate these diseases – and boost the economic value of their products.

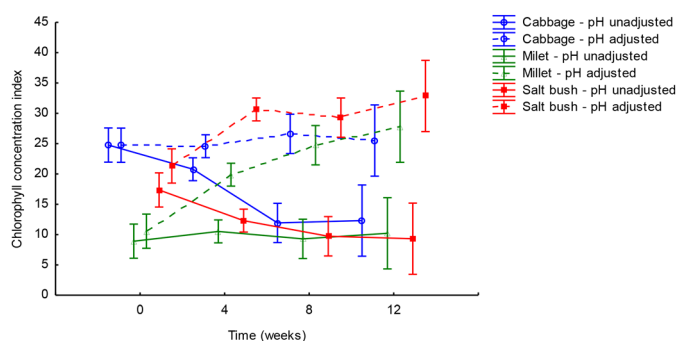
## THE INTERGRATION OF AQUAPONICS INTO A BREWERY EFFLUENT TREATMENT SYSTEM

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Brewery effluent (BE) is an organic effluent that contains valuable resources such as water and nutrients. Constructed wetlands (CW) treat BE to a standard suitable for aquaculture and crop irrigation at our experimental effluent treatment facility. There is a need to identify economically valuable crops, that can be used in place *Typha capensis*, to recover nutrients in BE before it is reused in down stream activities. The aim of this study was to determine the nutrient removal efficiency of commercial crops, the influence of pH on this efficiency, and the effect of alkalinity on crop growth and health when an aquaponic production system was incorporated into a brewery effluent treatment plant. Cabbage, saltbush and millet plants were grown in a recirculating hydroponic system fed treated BE that was either pH adjusted or pH left unadjusted.

The pH adjustment of post-primary facultative pond (PFP) BE had a major influence on the growth, health and chemical composition of plants grown in hydroponic systems. The chlorophyll concentration index of cabbage, saltbush and millet plants were all significantly higher when grown in the pH adjusted hydroponic systems (Repeated measures ANOVA,  $F_{(15,36)}=12.40$ ,  $p<0.0001$ ). The macro and micronutrient concentrations of cabbage leaves increased when the pH of post-PFP BE was adjusted to 6.5 at the start of each irrigation cycle. Post-PFP BE that was not pH adjusted was not a suitable water production of cabbage and millet plants as plants in these systems hardly grew. However, pH adjustment of BE renders it much more suitable for hydroponic/aquaponic crop production. The high alkalinity of BE is a major issue, firstly for decreasing the availability of nutrients in recycled effluent, and, secondly for making it hard to maintain a pH range of between 6.5 and 7.0 to optimise the availability of nutrients to the plants. Continual pH adjustment would increase the conductivity of the effluent, putting more osmotic stress onto the irrigated plants. The generation of alkalinity needs to be fully understood and technologies or practices need to be investigated that can reduce the alkalinity of the effluent. The pH plays a major role in the availability of nutrients to plants as well as influx/efflux of cations and anions through the plasma membrane. This needs to be further investigated because there is evidence that pH can influence the sodium efflux rate and sodium tolerance of plants.



**Figure 1** The mean ( $\pm$  95% confidence interval) chlorophyll concentration index of cabbage, millet and saltbush plants irrigated with post primary facultative pond effluent, over the 12 week experiment (Repeated measures ANOVA,  $F_{(15,36)}=12.40$ ,  $p<0.0001$ ).

## BIODIGESTION OF CULTURED UNICELLEULAR ALGAE

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Project Eden is an experimental treatment facility at Ibhayi brewery (Port Elizabeth, South Africa) where effluent treatment technologies that allow the reuse and recovery of water and nutrients are tested. High rate algal ponds (HRAP) that are used at the research site act as an alternative to activated sludge (AS) systems in conventional effluent treatment plants, due to their lower energy consumption. Our HRAP system has treated brewery effluent to the same quality and often more consistently, compared with post AS effluent. The treated effluent from the HRAP system is used in an aquaculture facility where catfish (*Clarias gariepinus*), tilapia (*Oreochromis mossambicus*) and guppies (*Poecilia reticulata*) are grown. There is a need to find an economic value for the algae that is cultured in the HRAP. This can be achieved by the conversion of algal biomass to methane during anaerobic digestion (AD). The methane produced can be used to run motors, pumps and heating facilities at the experimental treatment plant. This PhD aims to quantify the net energy balance of the HRAP followed by the AD of the culture algae and determine what culture conditions, pre-treatment procedures and co-digestion strategies can be used to optimise methane production during the AD of algae. The goal of this work is to identify water treatment processes that can produce energy to sustain the energy consumption of the treatment plant and water suitable for aquaculture.

The first experiment compared the water treatment efficiency and biomass production of HRAP and AS. An energy and carbon balance of activated sludge and high rate algal ponding is currently being conducted.

A second experiment evaluated the energy balance from the anaerobic digestion of HRAP and AS biomass. This was done by feeding five litre continuously stirred tank reactors for 90 days with algae and sludge feedstock from the HRAPs and AS respectively. Methane production rates and various water quality parameters were recorded to determine the performance and dynamics of the process. The energy and carbon balance of the anaerobic digestion of HRAP and AS waste biomass were determined.

Data for these first two experiments are currently being collected and will be presented at the conference.



## ECONOMIC VIABILITY OF SMALL-SCALE WARM FRESH WATER TILAPIA (*Oreochromis*), FARMING IN LIMPOPO PROVINCE, SOUTH AFRICA

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The study was conducted in Limpopo Province, South Africa, to assess the viability of tilapia farming. For this study a purposive sampling technique was utilized. It used non-parametric statistics, enterprise budgeting and the profit function model in data analysis transcribed into Microsoft Office Excel 2016 and IBM SPSS 22.0 software. Data was obtained from 17 out of 25 active fish farmers in the Province.

Socio-economic results reveal that tilapia farming is dominated by males between the ages 41-50, a greater portion of them with secondary education. Results indicated gross margin of R 15197.00, net farm income of R 6433.00, net return on investment of 0.76 and 0.30 rate of return on per capital investment (RORCI). This directly means that tilapia farming ensures a substantial level of profitability in the area of study. Training in fish farming and Cost of fingerlings positively and significantly influence return in fish farming, while Government support negatively yet significantly influenced return in tilapia farming. Predator, high cost of feed and lacks of financial assistance were the most reported or high ranked production constraint in tilapia farming in the study area.

It is therefore based on this background that government should play a central role by subsidizing farmers and putting in place a well-coordinated strategies exclusively for fish farmers' credit provision facilitation and training. For both income generation as well as food security purposes, unemployed women and youth within the active age group with access to water and land ought to be financially supported to be engaged in fish farming. For a much better profitability of the fish farming business in the study area, tilapia farmers need serious training in management aspects of fish farming including; site selection for pond construction, handling, record keeping and efficient use of resources.

**Table 1:** Average cost and return of tilapia production

| Item  | Amount (R)   | % TC       |
|-------|--------------|------------|
| TFC   | 8764.86      | 42         |
| TVC   | 12 303       | 58         |
| TC    | 21067        | <b>100</b> |
| TR    | 27500        |            |
| NP    | 6433         |            |
| GM    | <b>15197</b> |            |
| ROI   | <b>0.76</b>  |            |
| RORCI | <b>0,30</b>  |            |

**Table 2:** Distribution of the respondents according to the constraints in tilapia production

| Constraints | Frequency and percentage |           |             | Mean Rank |   |
|-------------|--------------------------|-----------|-------------|-----------|---|
|             | Not severe               | Severe    | Very severe |           |   |
| PDT         | 4<br>(23%)               | 1<br>(6%) | 12<br>(71%) | 1,47      | 1 |
| HCF         | 6<br>(35%)               | 0<br>(0%) | 11<br>(65%) | 1,64      | 2 |
| LFA         | 6<br>(35%)               | 0<br>(0%) | 11<br>(65%) | 1,64      | 3 |



## CHARACTERIZATION AND TRANSCRIPTIONAL ABUNDANCE UNDER PATHOGENIC STRESSES OF BIG BELLY SEAHORSE INTERLEUKIN- 10 (*Hippocampus abdominalis*)

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Interleukin-10 is known as anti-inflammatory cytokine that produced in T cells, B cells, eosinophils, epithelial cells, keratinocytes, macrophages, mesangial cells, tumor cells and NK cells. IL- 10 activates Jak-Stat signalling pathway, with the Stat3 by the ligated IL-10R complex. In this study, IL- 10 of big belly seahorse (*Hippocampus abdominalis*; *HaIL10*) was identified and characterized at sequence and transcriptional levels. *HaIL10* comprises coding sequence of 570 bp which encode respective protein with 189 amino acids in length. The HaIL-10 3D structure by homology modeling was similar to that of the human IL-10 monomer. Phylogenetic analysis revealed a close evolutionary relationship for HaIL-10 vertebrate counterparts, with close clustering to the fish homologs.

IL10 was expressed in all analyzed tissues with the highest in pouch then in muscle, while lowest expressions were observed in kidney and liver. The mRNA expression of HaIL-10 in intestine upon lipopolysaccharide (LPS) and poly (I:C) challenges shown a significant up regulated expression in first few hours and later down regulated only with poly (I:C) challenge. In addition, HaIL-10 expression has been increased significantly upon *E. tarda* and *S. iniae* challenges in intestine.

## **IS TILAPIA COMMERCIAL FARMING IN A RECIRCULATING AQUACULTURE SYSTEM A VIABLE PROPOSITION FOR THE EMERGING FARMERS (RURAL COMMUNITIES) OF THE VHEMBE DISTRICT OF LIMPOPO?**

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### **Background**

- Socio-economic issues confronting the communities of Vhembe District of Limpopo: unemployment of the youth, poor education especially in the trades, over-dependence on employment by employers for work opportunities.
- Fishing and fish eating culture in the communities.
- Factors that favour tilapia farming: government policies regarding emerging farmers, availability of manpower, favourable climatic conditions, availability of support for emerging farmers from ARC, U Limpopo, TAASA, DTI, DAFF. A huge demand for fresh tilapia of the “right standard” by very well organised retail chains, a strong veterinary science presence in RSA.

### **The Elim farm**

- Brief history – when established, costs, reaction of community to the project.
- First year of production; management, water quality issues, mortality statistics, system issues, first harvest, response of local community to our fish, TAASA support.
- Way-forward; increase in Elim Farm production towards 20 to 30tons /annum, ways to encourage growth of the industry in Vhembe District so as to qualify for a food processing plant and to achieve other benefits from economies of scale.

## RECIPIENT FISH AS MODEL ORGANISMS IN MULTILEVEL ASSESSMENT STUDIES

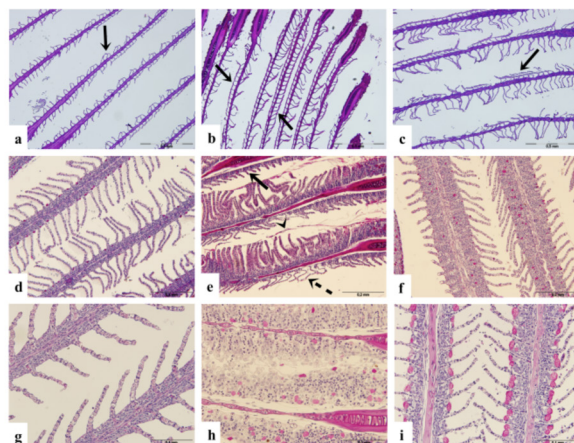
Natalija Topić Popović\*, Rozelindra Čož-Rakovac, Ivančica Strunjak-Perović, Josip Barišić

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Treated wastewater discharged from the wastewater treatment plant (WWTP) was evaluated by assessing its impact on downstream free-living fish (Prussian carp, *Carassius gibelio*) health status, microbiological contamination and antimicrobial resistance, fish tissue structure, blood biochemistry and erythrocyte nuclear abnormalities. Potential bacterial pathogens from fish and respective waters comprised aeromonads with a zoonotic potential. High resistance profiles were determined towards the tested antimicrobial compounds, mostly sulfamethoxazole and erythromycin. Histopathology primarily revealed gill lamellar fusion and reduction of interlamellar spaces of fish captured from the effluent. A significant increase in plasma values of urea, total proteins, albumins and triglycerides and a significant decrease in the activity of plasma superoxide dismutase were noted in carp from the effluent-receiving canal. A higher frequency of erythrocyte nuclear abnormalities was found in fish sampled from the effluent-receiving canal. Histological and haematological parameters differed significantly in effluent and downstream. Overall, treated wastewater modified the environmental parameters of the receiving surface waters, and played an important role in the antibiotic resistance scheme; the investigated histological, hematological and plasma biochemical parameters of fish indicated to significant changes related to a complexity of environmental stressors. The histopathological changes observed in gill tissues in particular could be related to xenobiotics in the effluent, microbial load on gills, impaired water quality and increased heavy metal concentrations and may serve as an important end-point in the discrimination between polluted sites.

There are potential public health concerns regarding aeromonad exposure amongst recreational fishermen who come into contact with fish inhabiting waters downstream from the WWTP, and WWTP workers who are occupationally exposed to wastewaters and their aerosols. A very high proportion of fish aeromonads demonstrated resistance, and a notable percentage of these exhibited a multiple resistance pattern. In this regard, negative effects of antimicrobial use on the environment, fish and human health should be elucidated, whilst more WWTPs should be involved in these complex studies.

Fig 1. Gill structure from Prussian carp in spring (a,b,c), summer (d,e,f), fall (g,h, i).



## A UNIQUE CONTINUOUS CONCENTRATION AND SEPARATION SYSTEM FOR LARGE WATER VOLUME REFINING

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One of the main challenges for the aquaculture industry is to concentrate and separate viruses, bacteria, microparasites, particles or microanimals from large production volumes. This is difficult when the production water is sea-water or very complex contaminated water. Typical filtration systems consist of a dead-end plate with more/less defined holes or passages in the range between 100 nanometres to several millimetres. The main challenge is related to specific species concentration, separation or sorting within large volumes. Normally the whole biomass is concentrated at one physical area without any form of intelligent separation or sorting. This greatly limits the application of filtration related to diagnosis, analyses and treatment of complex liquid.

The unique Continuous Concentration and Separation (CCS) technology presented in this abstract is to establish this unique separation and sorting in connection with the concentration. This technology should be able to improve the sensitivity and specificity of diagnosis and analyses significantly. In addition, the technology is able to remove, sort, separate all particles or cells within a small or large volume of complex liquid.

This first CCS unit were produced by using injection moulding. Using 9 Continuous Separation Unit (CSU) including 180 micrometres between the pillars, different kinds of liquid or water consisting of different kinds of micro-organisms have been tested. Different kinds of inlet and outlet pressure and regulation were used including different concentration of microorganisms.

The conclusion is that any microanimal including Salmon lice may be concentrated from large volumes of sea-water into very small volumes of homogenized concentrated lice. This will significantly increase the sensitivity of any sensor used on this small volume.

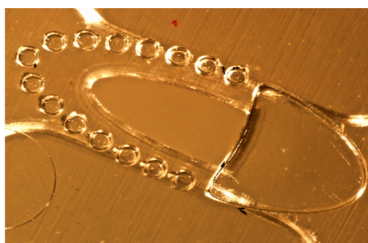


Figure 1: Microscope image of Continuous Separation Unit

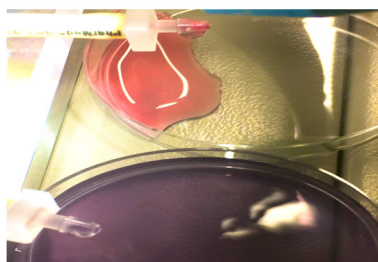


Figure 2: Concentrate outlet (upper tube) and Filtrate outlet (lower tube)

## **TRANSCRIPTOMAL CHANGES IN JUVENILE PRE-SMOLT ATLANTIC SALMON (*Salmo salar*) EXPOSED TO A CHRONICALLY ELEVATED WATER TEMPERATURE OF 20 °C.**

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<https://www.was.org/meetings/Abstracts/Default.aspx?code=WA2017>

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Atlantic salmon (*Salmo salar*) are a common aquaculture species. In Australia, Atlantic salmon production constitutes the largest commercial aquaculture sector. Globally, this industry has come under threat through increasing sea surface temperatures, predominantly during the summer months. Over the past 20 years, Tasmania has seen an increase in sea surface temperature off the eastern coast at a rate of 0.6 °C per decade. Currently, sea surface temperatures are ~18 °C during the summer months, however, they are predicted to rise by ~2 °C by 2060. The maximum recorded sea surface temperatures during summer is over 20 °C, which poses a significant risk to the Tasmanian Atlantic salmon aquaculture industry. This is primarily due to these temperatures exceeding their thermal optimum for growth, and may lead to chronic stress. Chronic stress is a maladaptive process brought about when the organism is in a prolonged stressful environment. It can adversely affect performance in growth, disease resistance, reproductive success, and survival. Yet little is currently known about this stress response in Atlantic salmon. The aim of this study was to subject Atlantic salmon to high water temperatures (20 °C) for a prolonged period of time (99 days), simulating the maximum temperatures that can be reached during the summer months. Using Next Generation Sequencing (NGS), we were able to demonstrate the differential expression profile of 348 genes. This resulted in the over expression of 358 biological processes, the significance of which will be discussed. Through this study we were able to demonstrate important genes that are sensitive in juvenile Atlantic salmon under a chronically elevated water temperature.

## A REVIEW: CHARACTERISATION OF MOZAMBIQUE TILAPIA (*Oreochromis mossambicus*) IN SOUTH AFRICA AQUACULTURE INDUSTRY

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The Mozambique tilapia (*Oreochromis mossambicus*) is a widespread indigenous fish species in South Africa. It is characterized as one of aquatic chicken genus and the improved strains entail a significant potential to supply high protein value to consumers at an affordable cost (Yosef, 2009). Introduction of exotic fish to South Africa, such as Nile tilapia has caused ecological disruption through habitat loss and extinction of endemic species including Mozambique tilapia. Therefore, genetic diversity of Mozambique tilapia populations worth to be conserved due to their unique qualities such as cold and salinity tolerance that might be valuable in commercial production. This led to consideration of environmental protection of local biodiversity by promoting Mozambique tilapia as a species that should be suited to small-scale aquaculture in South Africa.

To monitor the effect of the introduction of invasive tilapias, it is important to quantify genetic diversity of the native biota exotic tilapia. Genetic characterisation is the first step when making decision of gene pool conservation. The traditional method for characterisation was based on phenotypic traits using pedigree information. However, recent genomic approaches, such as genetic markers are important tools for measuring genetic diversity, extent of introgression, and estimating other genetic parameters. They also allow pedigree tracing and reconstruction, and the selection of animals with desired marker combinations. More accurate selection for genetic improvement of Mozambique tilapia can be exploited through these genomics applications (Jolly et al., 2010).

Currently, genetic characterisation of South African Mozambique tilapia has not been fully exploited. The recent advancement of high-density single nucleotide polymorphisms (SNP) assays have potential to reveal genetic variation within and between species. Therefore, the use of SNP markers to characterise Mozambique tilapia is recommended.



Figure 1: Mozambique tilapia



Figure 2: Nile tilapia



## THE RATE OF GROWTH AND PRODUCTION OF THE EARTHWORM *Alma millsoni* FROM CELLULOSE AND SOIL SUBSTRATES

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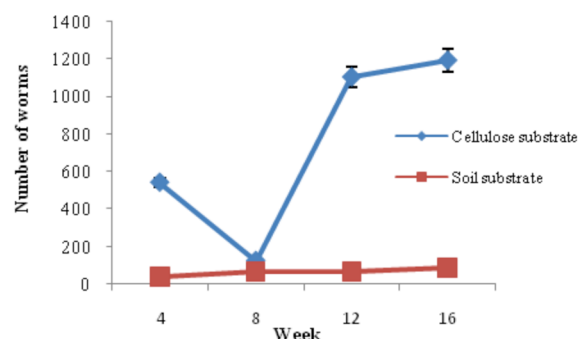
The earthworm, *Alma millsoni* was cultured in two substrates: cellulose and soil (control) in perforated plastic boxes (0.8m x 0.6m x 0.3m). The experiments were carried out in a randomized block design in triplicates for sixteen weeks. Thirty worms of weight  $2.01 \pm 0.25$ g and length  $22.01 \pm 4.06$ cm were stocked in each box. The worms were fed with dried poultry dung weekly at 10% of their body weights and sampled every two weeks. At the end of each month total harvesting of the worms was done in each box; the worms were counted, weighed and classified into their developmental stages by the absence, presence and size of the clitella. Temperature and pH of the substrates were monitored throughout the experimental period. The proximate and mineral compositions of the earthworms and substrates were analyzed before and after the culture using AOAC methods. Data collected were analyzed using descriptive statistics, ANOVA and t test; the level of significance was determined using Turkey Honest test. There was a general increase in the rate of growth with time for worms in cellulose substrate while for those in soil substrate, growth decreased after the twelfth week of culture; growth was better for worms in cellulose than soil substrate (Table 1, Fig.1). Generally, number of worms produced increased with time (Fig. 2). Biomass was higher ( $287.6 \pm 0.01$ g/20kg substrate) for earthworm cultured in cellulose substrate compared to those from soil ( $88.8 \pm 0.02$ g/20kg substrate).

Crude protein was significantly higher ( $61.79 \pm 0.15\%$ ;  $p < 0.05$ ) in worms cultured in cellulose substrate. Temperature values in the two substrates during experiment ranged from  $23^\circ\text{C}$ – $29.5^\circ\text{C}$  while pH values were  $6.02$ – $7.85$ . Temperature at highest production of the earthworms was  $25^\circ\text{C}$  and pH was  $6.92$ – $7.06$  in both substrates. Results from this study affirm that cellulose substrate is better than soil for the culture of *A. millsoni*.

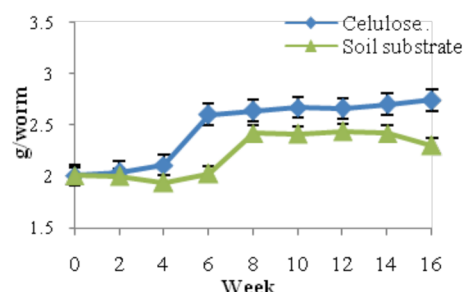
**Table 1: Growth performance and nutrient utilization of *Alma millsoni* cultured in different substrates**

| Parameters                            | Cellulose substrate | Soil Substrate     |
|---------------------------------------|---------------------|--------------------|
| Duration of Culture (weeks)           | 16.00               | 16.00              |
| No. of earthworms stocked             | 30.00               | 30.00              |
| No. of earthworms harvested           | $1190.0 \pm 5.00^a$ | $86.0 \pm 2.00^b$  |
| No. of worms harvested per week       | $74.0 \pm 5.00^a$   | $5.0 \pm 2.00^b$   |
| Initial weight of earthworms (g/worm) | $2.01 \pm 0.25^a$   | $2.01 \pm 0.25^b$  |
| Final weight of earthworms (g/worm)   | $2.74 \pm 0.02^a$   | $2.30 \pm 0.04^b$  |
| Mean weight gained (g/worm)           | $0.73 \pm 0.23^a$   | $0.29 \pm 0.21^b$  |
| Relative growth rate (%)              | $36.32 \pm 0.02^a$  | $14.43 \pm 0.02^b$ |
| Specific growth rate (%/day)          | $0.28 \pm 0.01^a$   | $0.12 \pm 0.01^b$  |

Values on the same row with different superscripts are significantly different ( $p < 0.05$ )



**Figure 2: Rate of production of *Alma millsoni***



**Figure 1: Growth pattern of *Alma millsoni***



## NUTRIENT COMPOSITION OF COMMERCIALY IMPORTANT FISH SPECIES OF THE MID CROSS RIVER FLOOD SYSTEM, SOUTHEASTERN NIGERIA

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Studies were conducted on the nutrient composition of commercially important fish species (*Tilapia zilli*, *Clarias gariepinus* and *Chrysichthys nigrodigitatus*) of the mid Cross River flood system, Southeastern Nigeria. The result obtained from the three sampled used shows that moisture has the highest percentage value followed by protein, carbohydrate and the least fat and oil. Among the three species used; *T. zilli* has nutrient composition of moisture to be: (79.4%), protein (18.0%), fat and oil (0.9%), crude fibre (0.8%), ash (0.6%) and carbohydrate (0.3%) which shows that moisture content was the highest with (79.4%) and the least was carbohydrate with (0.3%). *C. gariepinus* has the nutrient composition made up of (69.6%), protein (22.8%) crude fibre (3.1%), carbohydrate (2.1%) ash (1.5%), and fat and oil (1.0%) which shows that moisture has the highest value while fat and oil has the least value in that species. *C. nigrodigitatus* has nutrient composition of moisture to be: (73.1%), protein (24.5%), carbohydrate (1.4%), fat and oil (0.7%), ash (0.3%) and crude fibre (0.03%). In this species, moisture has the highest value while fat and oil has the smallest value, but in this study generally; *C. nigrodigitatus* has the highest percentage of protein among the three samples while *T. zilli* has the highest percentage of carbohydrate among the samples used. *C. nigrodigitatus* is however the best sources of protein and carbohydrate.

Table 1: Nutrient proximate analysis of *T.zilli*, *C.gariepinus* and *C.nigrodigitatus*

| Sample                   | Moisture | Ash  | Fat and Oil | Protein | Crude fibre | Carbohydrate |
|--------------------------|----------|------|-------------|---------|-------------|--------------|
| <i>T. zilli</i>          | 79.4%    | 0.6% | 0.9%        | 18.0%   | 0.8%        | 0.3%         |
| <i>C. gariepinus</i>     | 69.6%    | 1.5% | 1.0%        | 22.8%   | 3.1%        | 2.1%         |
| <i>C. nigrodigitatus</i> | 73.1%    | 0.3% | 0.7%        | 24.5%   | 0.03%       | 1.4%         |

## PHENOTYPIC AND GENOTYPIC CHARACTERISATION OF WILD AND CULTURE CICHLID POPULATIONS (*Tilapia guineensis*) IN LAGOS STATE, NIGERIA

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This study was carried out using phenotypic and genotypic approach in characterization and identification of *Tilapia guineensis* from the wild and culture. A total of forty (40) samples of *T. guineensis* were collected; twenty(10) samples each of farm-raised (Akinsateru and Nigeria Institute of Oceanography & Marine Research (NIOMR) and wild (Lagos and Badagry Lagoon) were collected in Lagos State for 3 months.

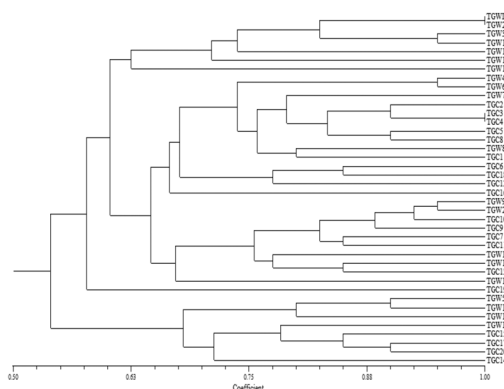
There were no significant differences ( $P \geq 0.05$ ) in all the phenotypic characters measured except at the Pre-Pectoral Length (PPL), Dorsal Fin (DF) and Weight (WEI) (Table 1). These could be as result of ecological factors such as temperature, pollution; management practices in the farm, etc.

The similarity coefficient at 50% and 78% shows that there is genetic variability amongst the wild and culture samples. The observed similarities in the banding pattern at 100% of the wild and cultured environment could be partly due to escape of the cultured into the wild during flooding of some farms or that farmers are collecting broodstock from the wild. This study provides a promising solution to DNA isolation using the more economical salting-out method for extraction and base-line data required for genetic manipulation of the selected population of the fish in aquaculture.

Table 1: Mean values of morphometric and meristic measurements were significant ( $p < 0.05$ ).

| measurements | WILD                           |                                 | CULTURED                          |                                 |
|--------------|--------------------------------|---------------------------------|-----------------------------------|---------------------------------|
|              | Lagos lagoon                   | Badagry lagoon                  | Akinsateru farm                   | NIOMR farm                      |
| PPEL         | 4.0 ± 0.33<br>(3.8 - 5.0)      | 4.6 ± 0.6<br>(3.1 - 5.1)        | 4.4 ± 0.33*<br>(3.8 - 5.0)        | 4.2 ± 0.56*<br>(3.3 - 5.0)      |
| DF           | 27.3 ± 0.67<br>(26.0 - 28.0)   | 27.7 ± 0.82<br>(26.0 - 29.0)    | 28.2 ± 0.42*<br>(28.0 - 29.0)     | 27.3 ± 0.48*<br>(27.0 - 28.0)   |
| WEI          | 82.0 ± 13.10<br>(63.1 - 100.8) | 98.20 ± 24.70<br>(70.3 - 129.6) | 93.7 ± 21.72*<br>(66.67 - 131.26) | 72.5 ± 36.66*<br>(26.0 - 128.7) |

Range in parenthesis. Values with superscript along the horizontal are significant at 0.05.



**Figure 1.** Dendrogram depicting the degree of relation between the wild and culture relationship between the wild and culture sample.

TGW1 - TGW10 = *T. guineensis* from the Lagos Lagoon  
 TGW11 - TGW20 = *T. guineensis* from the Badagry Lagoon  
 TGC1 - TGC10 = *T. guineensis* from the Akinsateru farm.  
 TGC11 - TGC20 = *T. guineensis* from the NIOMR farm

## AQUANURSERY: INNOVATION FOR *IN SITU* LARVAL REARING OF NEW SPECIES FOR AQUACULTURE

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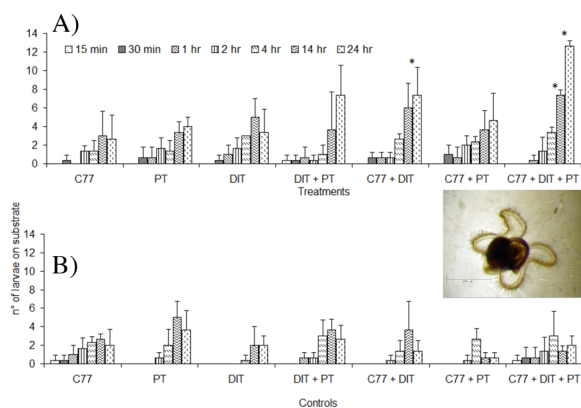
Why is it so difficult to develop new species for aquaculture? All of them share the same bottleneck: larval development. Raising new larvae species is complicated and expensive basically because we simply cannot feed them properly. Growing larvae food is expensive, technically complicated and nutritionally deficient, hence developing massive and profitable culture systems is unsuccessful. That is why we are creating the Aquanursery (patent pending), a product that will allow us to develop the culture of multiple new species for aquaculture. The Aquanursery is a device designed to take larvae to its natural habitat, rather than inefficiently try to replicate its conditions in a laboratory. By doing this we allow the larvae to naturally feed without extra costs, on its natural source of food using the ocean as an **ecosystem service**.

Aquanursery possesses a biofilm that naturally attracts the zooplankton inside, providing larvae with natural free food, making the production *in situ* of novel species for aquaculture possible.

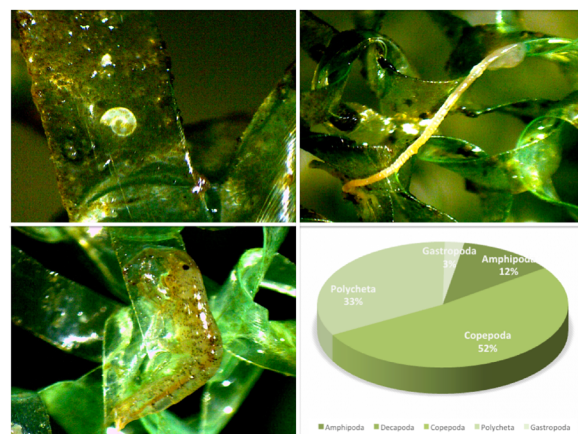
Preliminar experiments show us the capacity of the selected biofilm (C77+DIT+PT), developed over a inert substrate, to positive attract larvae of *Concholepas concholepas* (chilean Abalone) over it.

By the other hand, in other experiments where the biofilm over a inert substrate was exposed to natural conditions in sea water, the biofilm C77+DIT+PT present a better colonization of different and diverse zooplankton component.

These results suggest that larvae confined in systems with attractive biofilms would allow feeding of zooplanktonic food, allowing the production of juveniles in situ, with species without aquaculture developed technology.



**Figure 1:** A) attraction of the “chilean Abalone” larvae (60 DPH) to specific biofilms during 24 hrs of exposition v/s B) controls (substrates without biofilms). The film C77+DIT+PT present the most significant attraction to the substrates starting at 14 hrs post exposition.



**Figure 2:** inert substrates colonized with different taxa and grater number of individuals of marine zooplankton.

## APPLICATION OF BIODIVERSITY OFFSETS TO ADDRESS RESIDUAL IMPACTS FROM AQUACULTURE

Jan-Willem van Bochove\*, John Pilgrim, Suzanne Livingstone, Robin Mitchell

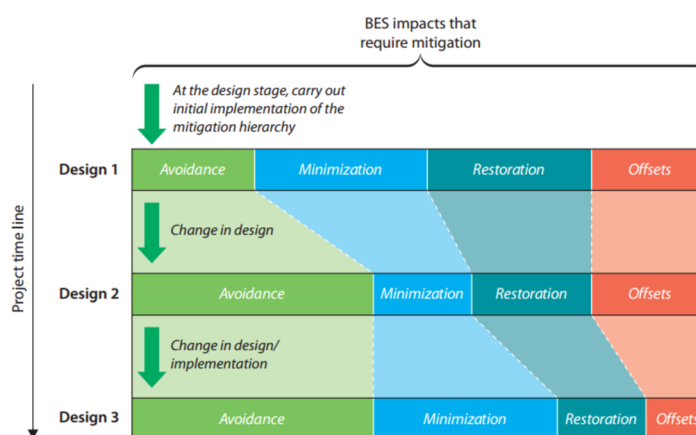
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Biodiversity offsets are measurable conservation gains that compensate for negative impacts of development projects that remain after full mitigation. Offsets should be seen as a last resort, following rigorous application of avoidance, minimisation and restoration measures as part of the Mitigation Hierarchy (see figure), with an emphasis on iterative redesign to minimise impacts and offset liabilities. Biodiversity offsetting generally aims to achieve no net loss (NNL) of biodiversity and, where possible, a net gain.

The potential impact of aquaculture projects on biodiversity is recognised as a significant business risk – and one that is attracting increasing attention from lenders. Offsetting is, for example, a requirement of the International Finance Corporation's Performance Standard 6 as well as the World Bank's Environmental and Social Standard 6. Offsets are also increasingly recognised by governments to reduce biodiversity impacts; more than half of the world's countries either have or are developing policies requiring the use of offsets.

Determination of offset feasibility and liability are most effective when explored as part of due diligence investigations or during the very early stages of project development, especially at the site selection and planning phase. Upfront screening is an effective means for determining potential impacts and identifying project alternatives that reduce or avoid the need for offsets altogether. Aquaculture offsets are likely to require landscape/seascape scale planning in order to be effective. Offsets within the aquaculture industry have seen very limited application but are likely to focus around:

- **Restoration** to remediate past damage, for example by rehabilitating or restoring degraded habitat elsewhere;
- **Averted loss** or protection offsets aim to prevent anticipated future damage, for example by preventing wetland conversion elsewhere;
- **Policy offsets**, aimed at changing policy and practice within the aquaculture sector, such as measures to enhance water quality elsewhere to compensate for project impacts.



*Figure 1: Sequential application of the Mitigation Hierarchy helps minimise impacts and offset liabilities through a focus on avoidance measures during the early project design.*

## **EFFECT OF ENVIRONMENTAL FACTORS ON SMOLTIFICATION AND POST-SALTWATER TRANSFER PERFORMANCE AND SURVIVAL OF FARMED TASMANIAN ATLANTIC SALMON *Salmo salar***

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The capability to migrate from freshwater streams out into saltwater environs requires juvenile salmonids to undergo extensive structural and functional changes in their physiology, morphology and behaviour. This crucial life phase is referred to as “smoltification”, or the parr-smolt transformation. In the wild, the change in salinity is gradual and salmonids can migrate out to sea when the time is right. On commercial salmon farms however, large numbers of fish are transferred directly from freshwater hatcheries to marine sea pens. All teleosts maintain a relatively constant internal osmotic pressure; around 300mOsmol/L. With freshwater being around 1-5mOsmol/L, and seawater around 1000mOsmol/L, these euryhaline fish must reverse their ion regulation methods during migration from what are primarily ion uptake mechanisms, to ion secretion mechanisms. It is therefore imperative that farmers understand the physiological changes that need to occur. Perhaps the most important of these is the development of an increased osmoregulatory ability, which can be observed via an increase in gill  $\text{Na}^+/\text{K}^+$ -ATPase activity.

Environmental factors such as photoperiod and water temperature are important stimulators and regulators of these crucial changes. Salmonid hatcheries can manipulate these factors in order to better prepare juveniles to be transferred from the freshwater hatchery tanks to the marine sea cages where they are grown out to harvest size. Fish that are poorly prepared for this extreme change in environment can show reduced growth, poor health and mortality post transfer.

We looked at farmed Tasmanian Atlantic salmon in situ over 2 consecutive years, and then compared these results to a laboratory based trial in an Aquaculture research facility at Deakin University. Fish were followed through their smoltification phase and then monitored post transfer. Different hatchery types (flow through vs recirculating water) showed different results in terms of development of osmoregulatory ability, and water temperature may also be having an effect. There was also a significant increase in mortality when fish are transferred to saltwater too early, as well as an increase in the occurrence of pin-heading syndrome, where fish cease to feed/grow after being transferred to sea.

## META-ANALYSIS OF DIGESTIBILITY STUDIES OF PROCESSED ANIMAL PROTEINS IN SEVERAL AQUATIC SPECIES COMPARED TO IN VITRO METHODS

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In aquaculture a lot of protein rich feed ingredients are needed. Therefore historically a lot of fish meal has been used in the formulas. Due to the expanding world population the need for aquaculture is still growing. As a result of this, the fish meal availability becomes a real challenge. Nowadays the search for sustainable alternative protein sources is a main research topic. Processed animal proteins of terrestrial animals are a logical source of proteins to focus on.

The advantage of those processed animal proteins are the usually high protein levels, containing the essential amino acids. When processing those animal proteins, several production techniques for heating and drying can be applied. All those different processing methods in combination with different animal tissues used as a raw material, result in distinguished digestibility figures of the valuable nutrients present.

To evaluate the nutritional quality of the processed animal proteins several in vitro techniques are available. Very often the standard AOAC<sup>1</sup>-method for protein digestibility is applied. With this method, based on the digestion in a pepsin solution, a very rough ranking of the nutritional value can be made. Next to this the so called Boisen<sup>2</sup> test is available. In this test two enzymes are used: pepsin and pancreatin. This test tries to mimic the real digestion in the intestinal tract. In practice both methods doesn't tell anything about the eventual process damage to the essential amino acids. For this reason also in vitro lysine availability tests have been developed. Like the method for lysine availability of Carpenter<sup>3</sup> and the guanidination method with *o*-methylisourea reactive lysine test<sup>4</sup>.

Of course the only real model is the animal model in the target species. To compare in vitro with in vivo results several processed animal proteins have been tested both in seabass<sup>5</sup> and trout<sup>6</sup>. Proteins tested comprises several blood meals, poultry meals and hydrolysed feather meals. Results will be shown to illustrate the deviation between the different in-vitro and in-vivo tests.

**Conclusion:** Based on the results found, the relation between in vitro and in vivo digestibility is not obvious. Both in vitro and in vivo models have their constraints. In the in vivo models used, the way of collecting the feces can significantly influence the results, whilst in vitro models always simplify the digestion processes.

## ENHANCING CLIMATE CHANGE RESILIENCE IN THE BENGUELA CURRENT FISHERIES SYSTEM

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The Benguela Current Large Marine Ecosystem (BCLME) spans some 30 degrees of latitude, extending from Angola's Cabinda Province in the north, to just east of Port Elizabeth in South Africa. It is one of the world's richest marine ecosystems and supports an abundance of life; sustaining both artisanal and large-scale fishery activities which contribute to local food security and employment for hundreds of thousands of people in areas of limited alternatives, and serve as important drivers of economic development. Climate variability and change form an additional challenge that could potentially push the natural ecosystem beyond its limits and the institutions established to manage human uses of and impacts beyond their capacity to do so. Impacts are already being felt through changes in surface water temperatures, an increased frequency of Benguela Niños and other such intrusions of warm, nutrient-poor water from southern Angola, an increase in winds in the summer months, a general decline in oxygen concentration, and sea level rise. Changes in the aquatic food web have also been observed, including distribution shifts in important fisheries resources with marked negative social and economic impacts. Angola, Namibia and South Africa have made significant efforts to address threats to the BCLME. These include the development and signature of a legally binding Benguela Current Convention.

A project, "Enhancing Climate Change Resilience in the Benguela Current Fisheries System", is a combined effort by the Benguela Current Convention, the governments of Angola, Namibia and South Africa, FAO and the Global Environmental facility (GEF). The goal of the project is to build resilience and reduce the vulnerability to climate variability and change of the marine fisheries and mariculture sectors within the BCLME through strengthening adaptive capacity and implementing participatory and integrated strategies in order to ensure food and livelihood security.

The project has been structured into three interlinked technical components:

- Component 1 which aims to ensure that national and regional policies and plans give due consideration, including well defined actions, to the likely implications of climate change and variability;
- Component 2 to put adaptation actions into practice in selected small and large scale fisheries as well as mariculture and communities in order to bring about measurable improvements in the pilots and to learn lessons for application on a wider scale; and
- Component 3 to increase both awareness and capacity to enable and promote a proactive, forward-looking approach to climate change.



## HATCHERY-JUVENILE PRODUCTION OF THE WEST INDIAN TOP SHELL, *Cittarium pica* (LINNAEUS, 1758)

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The West Indian top shell, *Cittarium pica*, is an endangered vetigastropod from the Caribbean. Due to widespread use of this snail as a food item throughout its geographical distribution, natural populations have declined substantially. Developing technology for conservation and repopulation of *C. pica* is therefore of interest as a tool to promote conservation. The aquacultural basis for production of juveniles in laboratory settings was developed and tested in this study. The spontaneous and/or induced spawning response of adult, wild-collected snails was evaluated under different conditions, moon phases and external stimuli (changes in temperature, salinity, desiccation, exposure to UV irradiated water and addition of Tris and hydrogen peroxide to the water). Development, growth and survival of embryos and/or larvae were measured under different temperatures (25, 27 and/or 29°C), embryonic densities (0.3, 0.6, 1.0, 4.0, 9.0 and 37.0% of bottom coverage), larval densities (0.5, 1, 1.5 and 10 larvae mL<sup>-1</sup>) and larval culture systems (static and down-welling). The growth and percentage of recovered post-larvae of 3 different batches were measured. Finally, early juveniles were cultured under different types of diets (fresh seaweeds, *Laurencia obtusa* and *Padina gymnospora*; natural multi-specific biofilm and *Cylindrotheca* sp. biofilm).

*C. pica* spawning responses were associated with daytime, flooding tides and new moon. Use of physical and chemical stimuli did not elicit the spawning response. Spawning adults maintained in the hatchery under reproductive conditioning for one year did not spawn again. Spawning of males and females were simultaneously and frequently intermittent. On average, males presented higher values of percentages of spawning and size than spawning females. The fecundity was positively correlated with total animal wet weight and the average value was 149,000 oocytes released female<sup>-1</sup>. Embryonic and larval development was reported in detail. Survival and growth rates of embryos and larvae were not affected by temperature, but higher growth and survival values were obtained at lower densities. Larval growth rate was not correlated with the type of culture system, but higher survival values were observed using the static system. Higher percents of recovered post-larvae (3%) were associated with lower densities of crawling veliger (0.15 larvae mL<sup>-1</sup>). Higher growth rate of post-larvae (27 µm day<sup>-1</sup>) was associated with the use of diatom biofilms with con-genera mucus as food and low water flow (50% h<sup>-1</sup>). No effect of type of food used was detected in the growth rate or survival of juveniles. In summary, we were successful in obtaining juveniles of *C. pica* in hatchery conditions, but additional research is necessary in order to improve the hatchery technology and to evaluate the use of juveniles hatchery-produced for aquaculture and/or restocking purposes.

## APPLICATION OF FUNCTIONAL METABOLOMICS TO IDENTIFY KEY METABOLIC CHANGES IN *Haliotis midae* DUE TO ENVIRONMENTAL HYPOXIA

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Oxygen is essential for normal metabolic processes of all organisms, and subsequently, many organisms have developed crucial process for survival when oxygen levels in the environment become depleted (hypoxia). Abalone have well developed mechanisms for surviving such conditions, however, any response to stress requires energy, which would be otherwise used for maintaining normal body functions like growth. Considering this, in the context of an abalone farm, hypoxia can occur due to handling practices associated with transport of the animals out of water. Generally the transition to hypoxia forces abalone to shift their metabolism towards anaerobic metabolism, which is not considered a sustainable source of energy production. Metabolomics is a functional tool being used to investigate complex interactions of metabolites. The aim of this study was to use a multiplatform metabolomics approach to evaluate which specific metabolic changes may occur in *Haliotis midae* as result of hypoxic incubation.

Muscle and haemolymph samples dissected from juvenile abalone subjected to hypoxia were prepared in accordance to the metabolomics method of interest. The analytical platforms used for analysis included: gas chromatography mass spectrometry, liquid chromatography mass spectrometry and nuclear magnetic resonance spectroscopy. Data extraction were performed using platform specific software followed by data analysis. Multivariate statistical methods were used to visualise natural grouping between the control and hypoxic group, and univariate analysis were used to identify compounds with significant differences between the experimental groups.

Principal component analysis score plots gave clear separation between the control and hypoxic groups (Fig.1), while *t*-test and effect size offered insights into the metabolites responsible for such differences. The metabolic pathways mostly influenced by hypoxic stress in muscle and haemolymph included: energy-, carbohydrate-, amino acid-, and nitrogen-, metabolism. By using a multiplatform metabolomics approach we were able to identify the metabolic changes taking place in *H. midae* due to hypoxia. Also, the end products of anaerobic metabolism namely: lactate, opines and succinate were increased in the hypoxic group, proving that abalone have well developed anaerobic pathways for energy production.

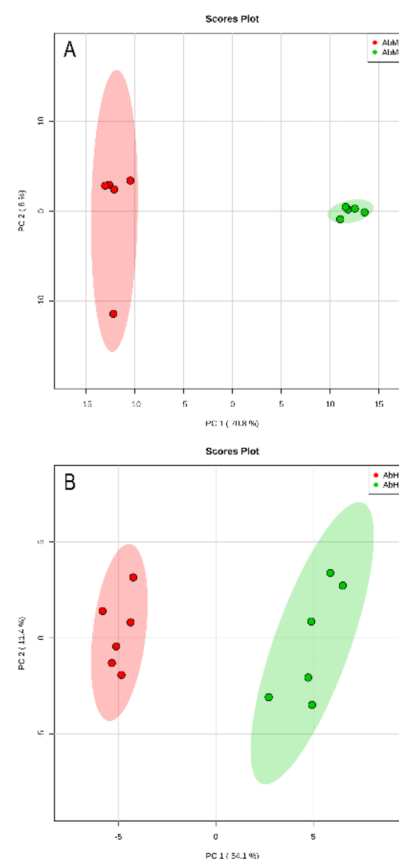


Fig.1 Principal component analysis score plot of statistically significant metabolite data of abalone A) muscle and B) haemolymph.

## MOVING SUBSISTENCE FARMERS TO COMMERCIAL FISH FARMING: DOES THIS EVEN MAKE SENSE?

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Governments, development agencies, NGO's, missions, often seek to use aquaculture as a development tool for a number of reasons. This paper discusses the premise for aquaculture as a development tool from the perspective of two production specialists who have worked in aquaculture since the 1970's. Table 1 shows potential yields and costs constructed using data generated over 20 years by the USAID-funded Pond Dynamics/Aquaculture Collaborative Research Support program (PDA/CRSP) in order to answer the question: can a subsistence farmer move to commercial feed-based fish production by reinvesting revenues generated from production to move up the technology ladder? It supposes that the pond already exists and the first stocking of the pond is done free of charge. The enterprise budget is very simple and covers only the inputs.

Table 1. Potential Yields and Costs of pond production based on management levels

This model helps farmers and planners understand the trade-offs in terms of investment and production. It allows them to foresee the cash needs for the next cycle of production. Development agencies that wish to assist small scale and subsistence farmers can gain some understanding of the types of assistance that can help. For example, if inputs are given for the first cycle, there has to be a plan to re-invest much of the sales revenue to purchase inputs for the next cycle.

| Level | Description of management                                      | Cash required for inputs | Cash generated from fish sales | Cash needed to finance next level | Cash left (return to labor, water, land) |
|-------|--|--------------------------|--------------------------------|-----------------------------------|--|
| 1     | On-farm inputs only; purchase seed only                        | \$80                     | \$150-300                      | \$138                             | \$12 - \$162                             |
| 2     | On-farm inputs, supplemented with chemical fertilizer          | \$138                    | \$300-450                      | \$243                             | \$57 - \$207                             |
| 3     | Bran 10cents/kg FCR3with chemical fertilizer 1.25bag           | \$243                    | \$900-1,200                    | \$538                             | \$362-\$662                              |
| 4     | Complete floating feed at half ration plus chemical fertilizer | \$538                    | \$1,050-1,350                  | \$538                             | \$512- \$812                             |

## TRAINING FUTURE COMMERCIAL AQUACULTURE MANAGERS THROUGH THE MASTER OF AQUACULTURE AT AUBURN UNIVERSITY

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The Master of Aquaculture (MAq) program began at Auburn University (AU) in the late 1970s in response to needs identified in some of AU's international programs as well as needs arising from the catfish farming industry which was developing in the Southeast United States. Over the years the program has had from 1 to 15 students at any time. A total of about 150 students have graduated from the program. The program is funded by revenues of fish sales, departmental operating funds, and occasionally from grants to support research activities.

The MAq is a non-thesis, production-oriented program. More coursework is required compared to the research-based Masters of Science degree, and an internship is required instead of a thesis. As a requirement of their assistantship, students are required to work on the E.W. Shell fisheries station to gain hands-on experience and learn the basics. Although the degree is meant to be terminal, some graduates have successfully completed a PhD program. Many graduates have multiple job opportunities in the private sector, extension services, state and federal hatcheries, university research stations, and community college and secondary education programs. Several have started their own commercial production farms.

The MAq students play a valuable role on Auburn's 800-hectare fisheries and aquaculture station assisting in the implementation of research programs, producing fish for use in research and for sale and operating a weekly fish market. They learn by doing, including activities like transporting fish for wholesale deliveries and conducting tours for school students and other public interactions.

Can this model be copied for other continents? Yes, but only if several factors come together at the same time. This presentation discusses some of the challenges faced by the MAq program and how it has evolved over the years. Alternative options for training fish farm managers are discussed.



## GLYCEROL CONTRIBUTION TO ENDOGENOUS GLYCOGEN SYNTHESIS IN THE EUROPEAN SEABASS *Dicentrarchus labrax*, AFTER A BOLUS INJECTION

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**Background:** The European seabass (*Dicentrarchus labrax*) is an important farmed marine fish species. As a mainly carnivorous fish, its metabolism is adapted to high levels of dietary protein, thus their efficiency in utilizing dietary carbohydrates is poor. Increased carbohydrate utilization would be both economically and environmentally beneficial, since high-cost fish meal could be substituted in part by lower cost substrates while at the same time the conversion of dietary amino acids to glucose and generation of waste ammonia would be spared. Glycerol is an important intermediary metabolite and a by-product of biodiesel synthesis and it has been evaluated as a feed supplement in rainbow trout and channel catfish. We hypothesize that glycerol effectively competes with dietary amino acids for gluconeogenic carbons thereby sparing their conversion to glucose/glycogen. As a first step, we determined if a bolus injection of [U-<sup>13</sup>C]glycerol would contribute significantly to hepatic glycogen appearance, as determined by <sup>1</sup>H and <sup>13</sup>C NMR analysis of hepatic glycogen <sup>13</sup>C-enrichment.

**Methods:** 20 fish (35.3 ± 3.8 g) were fasted for 21 days, anesthetized, injected intraperitoneally with 2 g kg<sup>-1</sup> [U-<sup>13</sup>C]glycerol dissolved in saline solution (0.9% NaCl). Fish were allowed to recover for 48h. After this period fish were first anesthetized and then sacrificed their livers excised for <sup>1</sup>H and <sup>13</sup>C NMR analysis. Hepatic glycogen was extracted, converted to glucose, derivatized to monoacetone glucose and analyzed by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy. The fractional contribution of [U-<sup>13</sup>C]glycerol to hepatic glycogen appearance was estimated from the excess <sup>13</sup>C-enrichment of glycogen carbon 1 as determined by the ratio of <sup>1</sup>H-<sup>13</sup>C- to <sup>1</sup>H-<sup>12</sup>C signals in the <sup>1</sup>H NMR spectrum.

**Results and Discussion:** From the <sup>1</sup>H NMR analysis, we observed well-resolved signals representing the <sup>13</sup>C-enriched glycogen that could only have been derived by gluconeogenic metabolism of the [U-<sup>13</sup>C]glycerol load. From the ratio of <sup>1</sup>H-<sup>13</sup>C- to <sup>1</sup>H-<sup>12</sup>C NMR signals, we estimated that 39.4 ± 14.9% of newly synthesized hepatic glycogen had been derived from the [U-<sup>13</sup>C]glycerol injection. This demonstrates that glycerol is avidly converted to glucose contributing to restore hepatic glycogen by european seabass, at least in the fasted state. Moreover, this capacity is constitutive since the fish were not previously fed with glycerol supplements.

**Conclusion:** European seabass are able to efficiently utilize exogenous glycerol for gluconeogenesis. This may represent a novel and economically viable strategy for minimizing the catabolism of dietary protein to glucose and glycogen in farmed fish.

## AQUATIC ANIMAL HEALTH MONITORING WITHIN THE BIOMARKER FRAMEWORK: FROM THE THEORETICAL TO THE PRACTICAL

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Although various definitions exist, in this presentation a biomarker will be considered as “a naturally occurring molecule, gene, or characteristic by which a particular pathological or physiological process, disease, etc. can be identified”.

The biomarker concept has wide application in medical and ecotoxicological fields. Here, I will argue that the biomarker concept can serve as a useful framework within which to assess and evaluate organismal responses to the unique stresses, processes and interventions of the intensive aquaculture endeavour. There exists the potential to use biomarkers for condition assessment, health assessment and as diagnostic tools to improve the farming effort.

Biomarkers differ in their specificity and biological relevance, and must be responsibly applied in order to allow appropriate deductions to be drawn about animal condition and health. To this end, biomarker responses need to be understood within the Janus context using increasing levels of biological complexity (Figure 1) and a *cause-effect-significance* scheme. A clear understanding of associative vs. cause-and-effect relationships between organismal responses and production end-points must be sought in order to meaningfully improve animal husbandry, system design or even feed development. To achieve this goal, responsible development (Figure 2) and application of biomarkers must be considered a priority for the aquaculture industry.

The need to understand the organismal stress response in the farming context is rooted in the energy partitioning between stress and growth. The stress response will detract energy and decrease the scope for growth of the individual organism, and this may eventually have a knock-on effect on the productivity of the farm and industry.

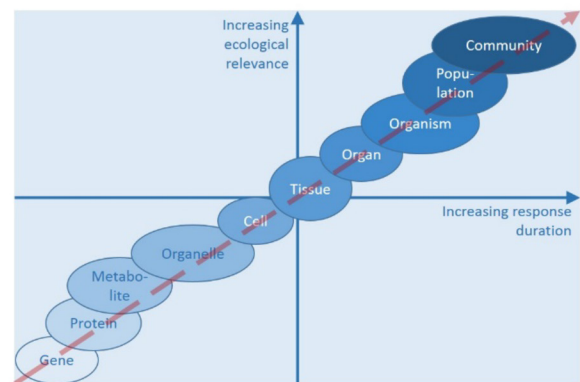


Figure 1 Biomarkers can be detected at different levels of organization, coinciding with different time frames for which the response will last

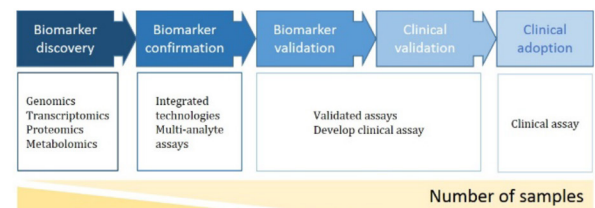


Figure 2 The development between biomarker discovery and eventual clinical implementation proceeds in a structured manner

## THE USE OF METABOLOMICS IN SOUTH AFRICAN AQUACULTURE

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The intensive culture of aquatic animals imposes stress beyond the capabilities of protective mechanisms endowed by their evolutionary history. The stressors include physical (e.g. water quality) and biological (e.g. crowding, parasites), some of which can be minimized through good husbandry practices.

The initiation and maintenance of protective responses to stress is energetically costly, and can shift the energy budget away from growth, affecting aquaculture productivity. It is essential that animal health be monitored routinely and deviations from optimal physiological conditions be identified accurately.

Assessment of aquatic animal health range from behavioural observations, histology, necropsy, haematology and metabolic end-points. Single metabolic end-points, e.g. blood glucose, can be informative, but lacks the context of the complexity of metabolic processes. Metabolomics has emerged as a more functionally relevant omics tool compared to transcriptomics and genomics, and has the potential to generate robust data describing animal condition from a metabolic perspective.

The promise and limitations of metabolomics as a tool in aquaculture will be explored through a series of case studies we have conducted on farmed fish and abalone from South African systems.



## DEVELOPMENT OF SEAWEED AQUACULTURE IN THE NORTHERN GULF OF MEXICO

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Seaweed production in the United States has gained attention among farmers and scientists alike due to its high nutritional value, bioremediation properties and potential for integration into multi-trophic aquaculture systems. While kelp farming is developing along the Atlantic and Pacific Coasts, little research has focused on open water cultivation of seaweed in the Gulf of Mexico. The northern Gulf of Mexico has potential to be an ideal location for seaweed aquaculture and could be integrated with existing oyster farms in protected coastal waters. In doing so, the integration of seaweed could benefit oyster farmers by providing a secondary cash-crop and benefit the ecosystem by reducing eutrophication in the surrounding waters.

To address the feasibility of seaweed farming in the northern Gulf, three species of macroalgae, *Ulva lactuca*, *Grateloupia taiwanensis* and *Chondria littoralis* were cultured on suspended long-lines in coastal Alabama and Florida at two different sites, between oyster cages and 30 m upstream from the cages. To prepare for grow-out of *U. lactuca*, an artificial seeding method was developed under controlled laboratory conditions to manage seeding density and biomass production. For artificial seeding, the collected thalli were first cut into 1 mm and 2.5 mm discs to induce zoospore release. Once a sufficient number of zoospores had been discharged, the suspension was added to prepared spools of polypropylene rope (24 m, 12 m/site) and cultured for 3 weeks to ensure successful attachment. *G. taiwanensis* and *C. littoralis* were prepared for grow-out by vegetative propagation of the thalli into equally sized fragments. The fragments were bundled at a density of 10 g, and spaced 10 cm apart on 12 m of polypropylene rope (6 m/site). Growth rates were determined by harvesting three meters of rope per species after four and six weeks in each season (summer, fall, spring). The cultured species were collected for tissue analysis and compared to water samples at the two sites to evaluate differences in nutrient uptake.

Trials during the summer and fall suggest that year-round cultivation of *U. lactuca*, *G. taiwanensis* and *C. littoralis* in the northern Gulf of Mexico is not feasible and is likely due to high water temperatures. Trials in spring 2017 are currently underway and results evaluating the growth potential of the species will be presented.

## MAPPING STUDY OF AQUATIC ANIMAL DISEASES IN SOUTHERN AFRICA

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The Fisheries Governance Project under AU-IBAR is strengthening institutional and policy environment for sustainable management and utilization of fisheries resources in Africa to improve production, food security and income. However, due to recent outbreaks of Trans-Boundary Aquatic Animal Diseases (TAADs) in Africa like Epizootic Ulcerative Syndrome, and lack of efficient and effective biosecurity control measures, the fisheries and aquaculture resources are at risk.

A rapid assessment was undertaken using SurveyMonkey Tool® to determine: i) status of aquatic animal diseases within region; ii) production systems (fisheries and aquaculture); iii) disease risks determinants; iii) the geographical areas where aquatic diseases have occurred. The purpose was to contribute to continental efforts in establishing biosecurity measures against the introduction and spread of aquatic animal diseases.

This region has experienced outbreaks of notifiable diseases namely EUS in Zambezi river basin, Koi Herpes Virus in South Africa and White Spot Syndrome Virus disease in Madagascar and Mozambique that have impacted on the livelihoods of communities dependent on this resource. Environmental factors and uncontrolled movement of live aquatic animals largely contribute to the introduction and spread these diseases.

Nevertheless, SADC has established a regional strategy that controls the spread of TAADS, and can be adopted for the Africa region. Furthermore, considerable information on parasites affecting marine and freshwater aquatic species has been generated through research. Member States will have to invest into human and infrastructural resources to strengthen capacity to control TAADS and emerging diseases.

## **SINGLE NUCLEOTIDE POLYMORPHISMS DISCOVERY IN THE TRANSCRIPTOME OF MARBLED LUNGFISH (*Protopterus aethiopicus*) BY NEXT GENERATION SEQUENCING: GUIDING BREEDING TECHNOLOGY**

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The marbled lungfish (*Protopterus aethiopicus*) is a potential aquaculture candidate in the East African region supporting livelihoods of many communities. There is limited genetic information on *P. aethiopicus* to guide its domestication. Therefore, Single Nucleotide Polymorphism (SNPs) molecular markers were developed for future use in selective breeding and genetic diversity programs. Genomic selection based on informative SNP markers would play a major role in the shift to appropriate breeding strategies. Total RNA was extracted from 18 marbled lungfish (3 fish per lake) specimens collected from Lakes Bisina, Edward, George, Kyoga, Nawampasa and Wamala. Through *de novo* assembly a total 6693 SNPs were identified. A total of 198 SNPs with a maximum heterozygosity value of 0.5 and flanking sequences of 140 base pairs of 40-60% Guanine-Cytosine content were selected. These were considered suitable to further guide in the aquaculture and conservation program of *P. aethiopicus*.

## DETERMINATION AND ANTIBIOTIC RESISTANCE PROFILES OF BACTERIAL PATHOGENS OF *Oreochromis niloticus* AND *Clarius gariepinus* IN UGANDA

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Bacterial diseases are a major constraint to fish production worldwide. Proper management of these diseases requires clear understanding of the prevalent etiological agents and available control options. In Uganda just like many other developing countries, there is limited reliable information about the prevalent species of fish bacterial pathogens. This study was carried out to establish the different species of bacteria infecting *Oreochromis niloticus* and *Clarius gariepinus* in Uganda and to determine their antibiotic susceptibility profiles.

A total of 288 fish samples from 40 fish farms and 8 wild sources were aseptically processed to isolate bacteria from internal organs (kidney, liver and spleen). Bacteriological swabs were also taken from the skin and any gross lesions observed. The samples were inoculated on Brain Heart Infusion agar (BHI) and/or Trypticase soy agar (TSA) together with selective agar plates for major tropical fish bacterial pathogens. The plates were incubated at 25- 30°C for 24 to 48 hours. Isolates were identified by their phenotypic and biochemical properties using conventional means and Analytical Profile Index (API) test kits (API20E and API 20NE). The antibiotic resistance profiles of selected isolates against fourteen (14) selected antibiotics were determined by Kirby-Bauer disc diffusion method.

Well-known fish pathogens were identified at a farm prevalence of; *Aeromonas hydrophila* (44%), *Aeromonas sobria* (21%), *Edwardsiella tarda* (8%), *Flavobacterium* spp. (4%), *Pseudomonas aeruginosa* (4%), *Pseudomonas fluorescens* (10%), *Citrobacter* spp (4%), and *Streptococcus* spp. (6%). Other identified pathogens with varying and unclear significance in fish production include; *Chryseobacterium indologenes* (13%), *Pseudomonas stutzeri* (2%), *Burkholderia cepacia* (2%), *Plesiomonas shigelloides* (25%), *Comamonas testosteroni* (8%), *Ralstonia pickettii* (2%), *Serratia marcescens* (4%), *Vibrio* spp. (10%), *Klebsiella* spp. (4%) and *Proteus* spp (6 %). There was a significant difference ( $P < 0.05$ ) in the occurrence of the bacteria according to production systems. Some bacteria species were highly associated with clinical disease in farmed fish. All bacteria evaluated for antibiotic sensitivity were sensitive to at-least ten (10) of the fourteen antibiotics tested. Some isolates expressed high levels of resistance to Penicillin, Oxacillin, Ampicillin, Clindamycin and Vancomycin, which is most probably intrinsic in nature, suggesting minimal or no acquired antibiotic resistance. To our knowledge, this is the first study to identify several of the different species of bacteria infecting fish and to determine antibiotic sensitivity profiles of fish bacteria in Uganda. This is vital information for fish disease management and provides baseline data for future reference. Characterization of the isolated bacteria is of great significance.

## ISOLATION OF ANTIBIOTIC RESISTANT *Tenacibaculum maritimum* STRAINS AND THEIR POTENTIAL AS LIVE ATTENUATED VACCINES IN OLIVE FLOUNDER

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*Tenacibaculum maritimum* (*T. maritimum*) is one of the most threatening fish bacterial pathogens that affect the culture of many marine fish species around the world. *T. maritimum* associated tenacibaculosis disease is manifested by ulcerative lesions on different parts of the fish body surface, including mouth, fin, tail, gills and eyes. Secondary bacterial and ciliated protozoan infections are also frequently associated, leading to high mortality rate of 90-95%. To date, the control of tenacibaculosis has been relying mainly on antibiotic treatment, which brings up the impact on marine environment. More effective and more environmentally friendly approaches to prevent tenacibaculosis need to be urgently developed.

In this study, we successfully isolated a number of mutant strains from wild type *T. maritimum* by repeated selection (over 20 passages) of antibiotic resistance on marine agar plates containing increasing concentrations of different antibiotics. The morphology and *in vitro* growth curve of mutant strains have been characterized. The TM-SP1 strain showed significantly longer cell length while the TM-NOV strain showed significantly slower growth rate compared to wild type and other mutants. The virulence tests for mutant strains were performed by intraperitoneal and intramuscular injections with different dosages in juvenile olive flounders. TM-NOV and TM-SP1 strains showed dramatically attenuated virulence compared to the wild type. The whole genome sequence comparison revealed that several key virulence factor encoding genes have been modified in mutant strains in comparison with the wild type. To examine the potential of two *T. maritimum* mutants as live attenuated vaccine, olive flounder juveniles were vaccinated with the live bacteria of two strains via intraperitoneal injection or immersion. Significant protection was found in the challenge experiment carried out at one month post vaccination. The serum antibodies against *T. maritimum* have also been detected by ELISA assay. Taken together, our results indicate that two *T. maritimum* mutants are virulence attenuated and can be used as a live vaccine for tenacibaculosis prevention.

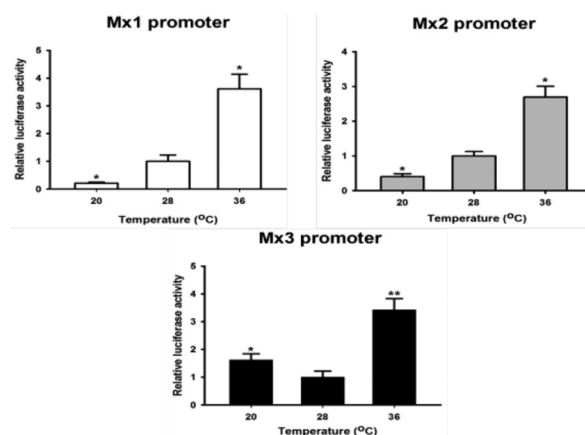
## TEMPERATURE MODULATIONS ON ANTIVIRAL RESPONSES IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

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Due to the impact of climate change, it can influence the rearing temperatures and pose a fundamental threat to the global aquaculture. As fish are poikilothermic animals, the innate immune systems can be deeply modulated by ambient water temperature. To assess the impact of temperature on antiviral responses of grouper, the interferons (IFNs) signal transduction pathway is applied to investigate the grouper antiviral responses at different temperature. This study will further provide information of the disease control strategy and development of fishery management under the impact of climate change.

In this study, we evaluated the antiviral responses in orange-spotted grouper at different temperature through investigating thermo- sensitivity of antiviral Mx protein. We firstly identified the regulatory motifs in three Mx promoters that contain different numbers of heat shock transcription factor 1 binding sites. To analyze the temperature effects on Mx promoter activities, promoter sequences were linked to a luciferase gene and the resulting constructions were transfected into grouper fin cells (GF-1 cells). Luciferase activity was measured post incubation at 20°C, 28°C and 36°C for 24 h (Fig. 1). It shows that elevated temperature can potentiate the activity of three Mx promoters differentially. Taken together, it suggested temperature modulation might influence Mx expression and lead to differential antiviral responses.



**FIGURE 1. Activity of Mx1, Mx2 and Mx3 promoters at 20°C, 28°C and 36°C.** GF-1 cells were transfected with each vector containing promoters and incubated at different temperature for 24 h. All results represent means and standard errors (n=4), relative to the group at 28°C. Values were compared using t-test (\*p < 0.05 and \*\*p < 0.01).

# THE IMPACTS OF SUSPENDED MARICULTURE ON COASTAL ZONES IN CHINA AND THE SCOPE FOR INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA), STARTING WITH THE NOBLE SCALLOP *Chlamys nobilis*

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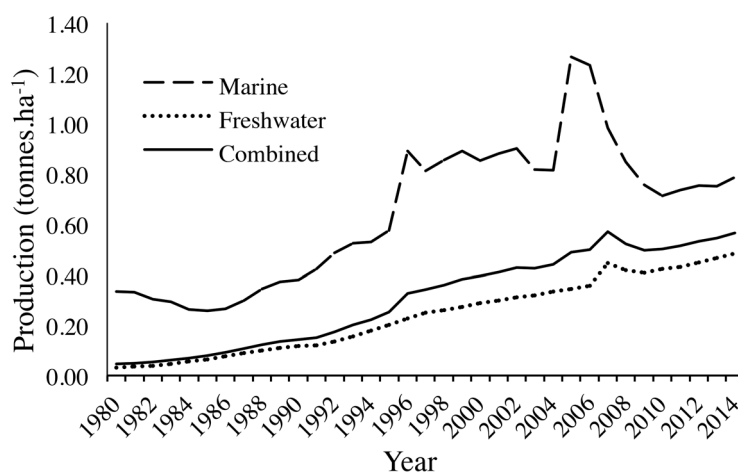
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Mariculture produced 38% of aquatic products in China in 2015. Although freshwater (FW) aquaculture still supplies the bulk of aquatic products, FW resources are under immense pressure and FW aquaculture produces less biomass per hectare than mariculture (Fig. 1). The dominant mariculture industry in China is suspended sea cage mariculture in the open ocean. One limitation to suspended mariculture is that it can impose several adverse impacts on the surrounding environment because systems are essentially open.

Since 1994, 162 papers have demonstrated eight chemical ( $n = 228$ ), five ecological ( $n = 64$ ), three anthropogenic ( $n = 12$ ) and two physical ( $n = 15$ ) impacts from suspended mariculture in China's seas. Of 92 papers that made explicit recommendations for improvements, 47 recommended either Integrated Multi-Trophic Aquaculture (IMTA) ( $n = 25$ ), large-scale bivalve production ( $n = 13$ ), or large-scale seaweed cultivation ( $n = 9$ ).

72 papers have investigated the applications of IMTA in Chinese coastal waters since 1996. 56 proved that IMTA could be used for biomitigation and 24 showed that IMTA systems improved farm production. Other potential benefits remain poorly studied. IMTA research papers are centralised in the Yellow Sea ( $n = 48$ ) whereas the Bohai Sea, East China Sea and South China Sea represent knowledge gaps.

As a case study, the commercially valuable scallop *Chlamys nobilis* was integrated into an inshore fish monoculture farm in Hong Kong in the South China Sea. The production potential and economics of *C. nobilis* integration with fish monoculture are presented.



**Fig. 1** Annual production (tonnes) per hectare from freshwater and marine aquaculture in China.



## THE EFFECT OF CARBON:NITROGEN RATE ON HETEROTROPHIC- AND NITRIFYING BACTERIA COMPOSITION OF BIOFLOC IN *Litopenaeus vannamei* SUPERINTENSIVE CULTURE

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There are two main groups of bacteria involved in nitrogen removal in biofloc technology culture systems (BFT). The absorption of inorganic nitrogen through heterotrophic bacterial growth promotes a faster reduction in ammonia concentrations than nitrifying process. The growth rate and bacterial biomass production of heterotrophic bacteria is 10 times higher than that of nitrifying bacteria. Autotrophic bacteria perform nitrification through the oxidation of ammonia to nitrite and then to nitrate. However, due to the slow growth of autotrophic bacteria, nitrite tends to increase significant in the environment culture. In this way, bacteria degrade excess organic matter and allow successive cycles of shrimp production without the need for water renewal culture. In general, organic fertilizations used to be done to increase the C:N ratio (15:1) for increase of heterotrophic bacterial population to convert inorganic nitrogen in bacterial protein. However, this growth of bacterial population used to increase the amount of total suspended solids (sludge) in shrimp culture, and sometimes can become a problem for BFT systems.

The aim of this study was to evaluate the effect of Carbon:Nitrogen ratio on heterotrophic- and nitrifying bacteria composition of biofloc in *Litopenaeus vannamei* superintensive culture in order to determine better bacterial composition for BFT system.

A 70 day-trial was conducted at the Marine Station of Aquaculture, University of Rio Grande, Rio Grande do Sul State, Brazil. After nursery, Pacific white shrimp ( $1.46g \pm 0.52$ ) juveniles were stocked at  $400/m^3$  in a twelve-1.000 L tanks. Four treatments (three replicates) were tested using four different C:N rates: 9:1, 11:1, 13:1 and 15:1. Water temperature, salinity, dissolved oxygen, pH, TA-N,  $NO_2$ -N,  $NO_3$ -N, Alkalinity and Settleable solids were daily monitored. BFT water samples were collected to detect the growth of the population of nitrifying and heterotrophic bacteria by Fluorescent *IN SITU* Hybridization determination - FISH. The results were analyzed by one-way analysis of variance (ANOVA) and Tukey's test ( $\alpha = 0.05$ ).

The water quality parameters were influenced by the treatments ( $P < 0.05$ ), but were not limiting for shrimp survival and growth in all treatments. Shrimp survivals were not influenced ( $P > 0.05$ ) by the treatments. The growth parameters were also different among treatments, ( $P < 0.05$ ), except FCR ( $P > 0.05$ ). The different C:N fertilization rate influenced significantly ( $P < 0.05$ ) bacterial population composition. Results showed the importance of decreasing the amount of Carbon to decrease total suspended solids in BFT system.

Acknowledgements: The authors are grateful to The Brazilian Council of Research (CNPq), FAPERGS, CAPES, Ministry of Fisheries and Aquaculture (MPA) – Brazil, Centro Oeste Rações S.A. (Guabi), All-Aqua Aeration, Aquatec, Trevisan and Inve do Brasil for funding this research.

## EXPERIMENTAL EVALUATION OF A HALOPHYTE *Salicornia virginica* FOR BIOMITIGATION OF DISSOLVED NUTRIENTS IN EFFLUENT FROM A RECIRCULATING AQUACULTURE SYSTEM FOR MARINE FINFISH

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The ability of a native salt marsh plant *Salicornia virginica* to serve as a biofilter for treatment of dissolved nutrients in effluent from a recirculating aquaculture system (RAS) for marine finfish was evaluated in the laboratory. The experimental objectives were to evaluate biomass yield and dissolved nutrient (N and P) removal capacity from marine RAS effluent of *S. virginica* planted in drainage lysimeters as a function of leaching fraction (LF), or proportion of water applied that leaches from the lysimeter after an irrigation event. *S. virginica* was planted in 20-L lysimeters housed in outdoor tanks with greenhouse enclosures. Raw effluent discharged from a pilot scale RAS producing black sea bass *Centropomus striata* was filtered (100  $\mu$ m) before use as an irrigant. Lysimeters were irrigated weekly with filtered RAS effluent (salinity = 29.2-38.6 g/L) to produce 30, 40 and 50% LF, with six replicate lysimeters per treatment.

Initial (d 1) above-ground biomass ranged from 10.2-12.1 g among the 30, 40 and 50% LF treatments, with no significant differences. Plant growth was excellent in all LF treatments through d 141 when growth plateaued in the 30% LF treatment (14.6 g), while continuing to increase in the 40% (19.1 g) and 50% LF (18.6 g) treatments. Net removal ( $\square$ g) of TN and TP by the plant-substrate lysimeter increased ( $P < 0.05$ ) with LF to a maximum at the highest LF (50%), with removal efficiencies of 53.9% for TN and 64.7% for TP. Compared to the irrigant salinity (29.2-38.6 g/L), leachate salinity in all LF treatments increased to 59.3-88.2 g/L by d 250, with higher salinities at lower leaching fractions, indicating that a buildup of salts in the lysimeter substrate over time was mitigated at higher leaching fractions. Excessive salt buildup in the root zone combined with high greenhouse temperatures in summer caused a decrease in plant biomass and shoot growth in all treatments after d 141, with plants irrigated at the lowest (30%) leaching fraction most adversely affected. By d 260, a clear trend toward greater plant biomass with higher LF was evident, with significantly higher biomass in the 50% (13.8 g) and 40% LF (12.0 g) treatments than in the 30% LF (3.88 g) treatment. Consistent with this trend, net removal ( $\square$ g) of TN and TP increased with increasing LF to a maximum at the highest LF (50%), with removal efficiencies of 56.7% for TN and 71.6% for TP.

The results demonstrated that effluent from a RAS for marine finfish promoted growth of *S. virginica* in drainage lysimeters. A leaching fraction of 30% was deficient, while leaching fractions of 40% and 50% were more effective in minimizing salt buildup in the root zone and maximizing nutrient removal from the irrigant and plant growth, but higher leaching fractions merit evaluation. The results suggest that, applied in combination with a Geotube for solids removal from raw effluent from a RAS for marine finfish, integrated multi-trophic aquaculture using *Salicornia virginica* may be a practical means for biomitigation of dissolved nutrients in the Geotube filtrate to render the treated effluent potentially safe for reuse in the RAS, or for release to the environment.

**WITHOUT GOSSYPOL, COTTONSEED PROTEIN SUBSTITUTES FOR FISH MEAL IN DIETS  
FED TO SHRIMP *Litopenaeus vannamei* BLACK SEA BASS *Centropristis striata* POMPANO  
*Trachinotus carolinus* AND FLOUNDER *Paralichthys lethostigma***

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Many plants that exist on the planet today are here because they have evolved a chemical defense mechanism that limits predation. Cotton has evolved the anti-herbivory toxin “gossypol”. A recent breakthrough, using modern molecular biology, virtually eliminates gossypol from cottonseed. Worldwide adoption of this technology will eventually make available more than 10 million metric tons (MMT) of highly palatable cottonseed protein, equivalent to about 16 MMT of fish meal. Cottonseed protein flour (more than 50% crude protein), both non-GMO “glandless” and the GMO version have recently been evaluated in diets fed to shrimp, pompano, black sea bass and flounder. Without exception, cottonseed protein with added Lysine was successfully substituted for most or all of the fish meal in the diets without affecting performance. A review of the recent research will be presented.

Shrimp (Samocha et.al.)

Previous studies have shown that glandless cottonseed meal (GCSM) can effectively replace 67-100% of fishmeal in shrimp diets without any negative effects. This study evaluated GCSM and two transgenic cotton lines with ultra-low gossypol (ULGCS) cottonseed flour as a replacement for fishmeal in a 64-day growth trial (35% crude protein diet). No significant differences were found between treatments in terms of final weight, survival, growth, and feed-conversion ratio. These results suggest that GCSM and/or transgenic ULGCS meals and flours can be used to replace fishmeal in commercial shrimp diets.

Juvenile Black Sea Bass and Flounder (Watanabe et.al.)

No significant differences were observed in survival, feed utilization, digestibility or weight gain among fish fed the control or test diets after 16 weeks, indicating that 100% fish meal could be replaced by glandless cotton protein in the diet of juvenile black sea bass. No significant differences in survival, growth, feed efficiency and body composition were observed in fish fed diets replacing up to 75% fish meal protein with either glandless cottonseed protein or ULGCS in flounder.

Pompano (Davis et.al.)

A basal diet (soybean meal and poultry by-product meal) was modified by replacing soybean meal with various cottonseed flours. The 10-week feeding trial indicated that the performance of fish offered the basal diet or diet containing glandless cottonseed flour performed better than fish on the other two cottonseed meals containing gossypol. The glandless cottonseed meal diet also had the highest energy and protein digestibility. The results confirm that GCSM would be considered a suitable protein source for Florida pompano.

## **PARTIAL SUBSTITUTION OF FISH MEAL BY A MIX OF ANIMAL AND VEGETABLE FEEDSTUFF IN RAINBOW TROUT *Oncorhynchus mykiss* IN ARGENTINA**

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Results of rainbow trout growth with fish meal replacement by a mixture of different ingredients are presented. The composition of the control diet (C) was fish meal (30%), sunflower meal (33%), meat meal (15%), corn (11%), fish oil (5%) and premix (1%). Treatment A (TA) had a 50% replacement of the fishmeal by a mixture of ingredients composed of blood meal (15%), poultry by products meal (20%), corn gluten meal (12%), beer yeast (33%), and egg albumin (10%). Proximate analysis of the mixture showed 54.6% crude protein, 5.26% lipids and 6.5 % moisture. Treatment B (TB) had a replacement of 75% of the fish meal by the same mixture. Used foods analysis showed an increase of protein as the replacement increased, resulting from 41.4% for the control, 41.6% for treatment A and 42.3% for treatment B. Experiments were carried out in CENADAC (23 ° 32 S, 58 ° 30W) in 1 m<sup>3</sup> glass fiber tanks for 130 days and each treatment had three replicates. Twenty fish per tank were placed with aeration and continuous water replacement. Water mean temperature during the cycle was 20.9° C; dissolved oxygen concentration 7.9mg / L and mean pH value was 7.6. The average fish weight at the beginning of the culture was 44g (TA), 42.2g (TB) and 50.1g (C); Average weights at the end were 187.4g; 184.9g and 193g respectively. The daily weight gain (DG) was similar with values of 1.1g / day (TA), 1.09g / day (TB) and 1.09g / day (C) ( $p > 0.05$ ). Food Conversion Ratio (FCR) were 1.77 (TA), 1.72 (TB) and 1.89 (C) without significant differences, as well as the protein efficiency ratio (PER), which showed values of 1.54 (TA), 1.56 (TB) and 1.52 (C), showing that it is possible to partially substitute fish meal in this stage of culture. A blend of ingredients of different origins and high protein quality is appropriate for this stage of rainbow trout farming. A complete replacement of the fishmeal should be tested, as well as continuing the experiments until the full culture cycle is completed.

## COMPARATIVE STUDY OF GROWTH PERFORMANCE OF *Heterobranchus longifilis* REARED WITH TWO ORGANIC FERTILIZERS IN EARTHEN PONDS

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The performance of *Heterobranchus longifilis* reared in earthen ponds fertilized with two organic fertilizers (meadow grasses and chicken droppings) was investigated. The study was carried out in 6 earthen ponds measuring 24.9 meters each at Nigerian Institute for Oceanography and Marine Research (NIOMR) fish farm Ijoyi-Badore Lagos.

Results obtained showed better food conversion ratio value in treatment 1 ( $1.28 \pm 0.11$ ) fertilized with meadow grasses and chicken droppings in combination with commercial extruded feed than in treatment 2 ( $1.72 \pm 0.32$ ) fertilized with only chicken droppings in combination with commercial extruded feed.

The total weight gain was  $665.39 \pm 22.05\text{Kg}$  and  $623.93 \pm 40.64\text{Kg}$  respectively for treatment 1 and 2 while the daily growth rate was  $4.01 \pm 0.13\text{g}$  and  $3.76 \pm 0.24\text{g}$  for treatment 1 and 2 respectively. *H. longifilis* in treatment 1 performed better than treatment 2 probably due to the efficiency of the organic fertilizers in treatment 1 than treatment 2. Fertilizer used in treatment 1 could be used in fish ponds to reduce cost of pond fish production.

(Continued on next page)

Table 1: Mean values of the growth parameter of the treatments

| Growth Parameters        | Treatment | Mean values $\pm$ S.D. |
|--------------------------|-----------|------------------------|
| Food Conversion Ratio    | T1        | $1.28 \pm 0.11^b$      |
|                          | T2        | $1.72 \pm 0.32^{ab}$   |
| Weight Gain (kg)         | T1        | $665.39 \pm 22.05^a$   |
|                          | T2        | $623.93 \pm 40.64^a$   |
| Daily Growth Rate (g)    | T1        | $4.01 \pm 0.13^a$      |
|                          | T2        | $3.76 \pm 0.24^a$      |
| Survival Rate (%)        | T1        | $96.73 \pm 0.70^a$     |
|                          | T2        | $93.47 \pm 1.40^a$     |
| Mortality Rate (%)       | T1        | $3.27 \pm 0.70^a$      |
|                          | T2        | $6.53 \pm 1.40^a$      |
| Specific Growth Rate (%) | T1        | $3.54 \pm 0.07^a$      |
|                          | T2        | $3.52 \pm 0.04^a$      |

Means of the growth parameters followed by different letters as superscript are significantly different between and within the treatments ( $p < 0.05$ ). Means values are followed by  $\pm$  S.D.

Table 2: Nutrient utilization by experimental fish samples Parameters

| Parameters                                  | Trt 1   | Trt 2   |
|---|---------|---------|
| Protein Intake(g)                           | 355.865 | 472.659 |
| Protein Efficiency Ratio (PER) (g)          | 1.870   | 1.321   |
| Apparent Net Protein Utilization (ANPU) (%) | 0.953   | 0.791   |



Plate 1: Meadow grasses used for the hay



Plate 2: Inside view of the ponds layout



Plate 3: *Heterobranchus longifilis* specie

## **NATIONAL COUNCIL OF SPCAS: MANDATE AND EXPERIENCE RELATING TO AQUACULTURE**

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The National Council of SPCAs (NSPCA) was founded over 60 years ago. We prevent cruelty, promote kindness and alleviate the suffering of animals.

The SPCAs in South Africa are governed by the SPCA Act 169 of 1993 which is administered by the NSPCA, constituting us as a statutory body. Inspectors are authorised in terms of the Animals Protection Act 71 of 1962 with the SPCA movement collectively undertaking over 90% of all animal welfare investigations and prosecutions in South Africa.

The NSPCA operates on a national basis with a team of dedicated Inspectors, who are specialists in their fields. We work tirelessly to protect animals from neglect and abuse and enforce the Animals Protection Act 71 of 1962 when deliberate cruelty takes place. The lives of tens of thousands of animals are improved because of our passion, our intervention and our commitment to bring about long-term positive change for all animals.

In a country that spans over a million square kilometres, there are areas where there is no SPCA, no animal welfare organisations and no veterinary services. The NSPCA plays a vital role in bringing extraordinary support to these people and their animals.

The NSPCA is in the process of developing welfare standards and codes of practice to reduce stress and pain. Although the same concerns and welfare principles apply as with other food animals, we are faced with an additional challenge, since this is a vast and varied field, covering abalone, shellfish and various freshwater and sea fish species. Currently, as South Africa is signatories for the OIE, we need to comply with their standards or practice even better animal welfare. In this presentation we will elaborate on this and include scenarios that have been experienced by ourselves.



## REARING THE CALANOID *Acartia tonsa* Dana AND THE HARPACTICOID *Tispe holothuriae* L. IN MIXED CULTURES AS LIVE FEED FOR FISH LARVAE – DO THEY POSE COMPETITIVE EXCLUSION OR CO-EXIST?

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Mono species live feed copepod cultures are always at risk for contamination. When using natural seawater one has only limited control of the microbial community, proto- and metazoans including copepods. In a meso-scale recirculated aquaculture system (RAS) rearing the holoplanktonic calanoid *Acartia tonsa* at 17 °C in dark we have experienced frequent contamination by a harpacticoid copepod, *Tispe holothuriae*. *Tispe* is during adulthood surface associated and during larval stages pelagic, i.e. semi-benthic. The contamination likely stems from an integrated algal culturing facility, a photo bio reactor (PBR), where its presence is tolerated since it to certain extend control biofilm formation at the surfaces in the PBR. Since our purpose is to culture *A. tonsa* and to harvest eggs for storing, shipping, and use the hatched nauplii in marine fish hatcheries we need to clarify if the two copepod species pose competitive inclusion or co-exist in cultures. This is indeed important when it comes to the scheduled end-product, a cold stored clean *Acartia* egg product. Therefore we conducted two 40 d competition experiments in 3-4 replicate 2200-2400 ml experimental containers with food in excess (*Rhodomonas salina*). Either a monoculture of 160 ind. of one or the other species or an equal density of 80 ind. of each of the two species was inoculated and population(s) development was followed. Firstly we established a setup with limited turbulence in beakers with stagnant water and gently aeration, expected to favor the semi-benthic *Tispe*. Secondly we established a setup with gentle turbulence in closed topped up bottles mounted on a slowly rotating plankton wheel (0.5 rpm), expected to favor the holoplanktonic *Acartia*. Indeed our expectations hold true, but a co-existence at very different population levels of the two species was demonstrated for both setups. To investigate if *Tispe* predate on *Acartia* eggs we conducted a 24 h experiment in stationary 6-well trays each containing 3 ml of filtered (2 µm) seawater. Ten non-starved female *Tispe* with egg sacks were collected and placed separately in 10 wells. An aliquot of 20 *Acartia* eggs and no algal food were added to each well. No egg predation was registered. Finally we have to ensure that in case *Tispe* eggs or nauplii contaminate the *Acartia* eggs harvested from our cultures they cannot find their way to the final *Acartia* egg product. We harvested eggs from one of our *Acartia/Tispe* mixed copepod culture tanks, rinsed them, and hatched in filtered seawater for 72 h in 10 replicate Petri dishes with approximately 200 eggs in each. We obtained a hatching success of ~85 % of solely *Acartia* nauplii. As opposed to the free spawning *Acartia* copepods *Tispe* is an egg carrier and hatches their nauplii directly from their egg sacks leading to a negligible risk for egg contamination at the bottom of the culture tanks. Hence, even though *Tispe* is a contaminator in an *Acartia* culture the two copepod species can co-exist without *Tispe* influencing our end-product. Moreover, *Tispe* might even be advantageous since it cleans surfaces for biofilm.

## **STRONGER TOGETHER – DEVELOPMENT AND IMPLEMENTATION OF COLLABORATIVE POST-GRADUATE PROGRAMMES IN AQUATIC SCIENCES, AQUACULTURE & FISHERIES**

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The African Union and international agencies consider capacity building in the fisheries and aquaculture sector as a crucial factor to develop Pan African policies and to implement innovation/transformation processes for the sector development. Institutions of Higher Education & Research are considered as key-agents for innovation, transformation and the provision of highly qualified professionals. Following the massive student enrolment expansion and the establishment of a large number of new universities with limited capacity in several African countries during the last decade, the issue of academic quality has become an issue of major concern. The lack of highly qualified staff, adequate teaching/research infrastructure and regional/international networking were identified as the most critical factors to affect the quality of university education and hence fit-for-demand graduates negatively. These factors are highly relevant for academic programmes in fisheries and aquaculture, since quality education in these sectors require laboratories, field-research facilities and practical courses to generate highly skilled graduates.

Collaborative academic programmes offer enormous potential to uplift the quality of education and graduates and to initiate transformation processes, such as:

- Combining complementing expertise and equipment/laboratory/field-facilities of several institutions;
- Delivering international experience and intercultural competence via student and staff mobility;
- Provisioning of excellent platforms for academic staff mobility, regional/international networking and development of joint research/development projects;
- Facilitating the integration of non-universities into academic programmes, such as research/management institutions, NGO's and the private sector;
- Stimulating research and on regional challenges/solutions, such as transboundary- and Pan African issues and contributing to the development/ implementation of evidence based regional policies;
- Supporting regional/international harmonisation processes in the higher education sector (credit transfer, quality assurance procedures, etc); and
- Contributing to the achievement of SDG's 2, 4, 8, 10, 12, 14 and 17.

Discussed are the benefits, challenges and recommendations for planning and implementing collaborative academic programmes, based on the practical experience of several international joint/double degree programmes with the involvement of the University of Natural Resources and Life Sciences, Vienna and African institutions.

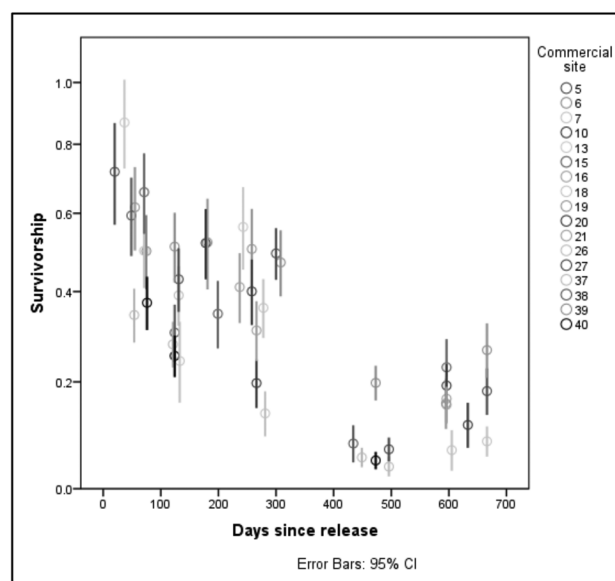
## ABALONE STOCK ENHANCEMENT AT CAPE RECIFE, THE EASTERN CAPE PROVINCE OF SOUTH AFRICA

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South Africa has legislated a policy of abalone ranching and stock enhancement as a potential solution to rebuilding abalone stocks depleted by illegal fishing. Experimental ranching rights have been issued to private entities who are required to work with research service providers to undertake seeding and performance monitoring. A ranching pilot study was undertaken where small-scale replicated seeding experiments were set up which quantified the effect of seed size, density and habitat on survival and growth of hatchery-reared abalone. 36 experimental plots were seeded with 8400 abalone spread evenly over two broad habitats. Mean seed retrievals were 17% ranging from 4 – 42% after a minimum of eight months at large. Release density and large-scale habitat had no significant effect on seed survival, but smaller seed size ( $40 \pm 3.7\text{mm}$ ) achieved higher retrievals than larger seed size ( $53 \pm 3.7\text{mm}$ ). Growth rates were an average of 30% higher in the shallow sites compared deeper sites.

Abalone seed releases were also conducted on a large scale, which focused on the economic potential for commercial-scale ranching. Over 160 sites were seeded with 1.4 million hatchery reared abalone or 30 tons. 17 sites underwent long-term monitoring of survival with repeated sampling events on sites over a period of up to 666 days. Nine of these sites were seeded with 140800 small abalone (29 – 40 mm), three sites with 25100 medium abalone (30 – 46 mm) and five sites with 14700 large abalone (60 – 84 mm). There was a significant difference in survival between the seed sizes. The generalized linear mixed model calculated the instantaneous apparent mortality rate for small, medium and large abalone at  $1.12\text{ yr}^{-1}$ ,  $-0.9\text{ yr}^{-1}$  and  $-0.67\text{ yr}^{-1}$  which equated to a survival rate of 21%, 28% and 36% a year respectively. In conjunction with deep-water somatic growth rate of  $1.2\text{mm.m}^{-1}$  and plausible emigration rates, a range of biomass harvesting estimates were obtained. Movement and mortality were investigated using time-lapse analysis which showed that hatchery seed had the ability to move considerably once released onto natural reefs and confirmed that mortality immediately following releases was due to the heavy predation pressure as confirmed by shell collections. This project has demonstrated that potential exists for ranching in South Africa and highlights critical factors that need to be addressed if ranching is to be an economically viable operation.



**Figure 1** Mean survivorship of all abalone size classes for 17 sites monitored at varying intervals for a period of up to 666 days post release.

## MULTI-CRITERIA FUZZY DECISION SUPPORT SYSTEM (MCFDSS) FOR ABALONE RANCHING PLOT SELECTION

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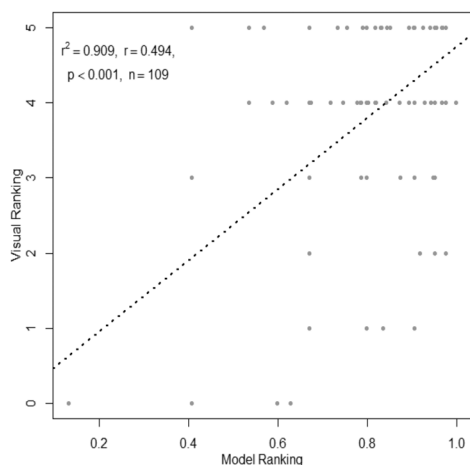
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South African government has launched operation “Phakisa” which among other things has emphasised the development of the aquaculture sector in the country. One of these projects recognised under this initiative is the commercial abalone ranching operation established at Cape Recife, Port Elizabeth, South Africa. Abalone ranching and stock enhancement can be described as the processes of releasing hatchery bred stock onto the natural reef systems, with intention of harvesting a designated tonnage of these animals in the future, while maintaining and strengthening the natural population. The main aim of this research was to contribute to this, by creating a system that could assist in the selection of suitable ranching sites, and improve the management of the ranching program.

An unsupervised classification approach was used to group the colour signatures of pixels in satellite imagery of the ranching areas. An initial 30 signature classes were identified and subsequently reclassified into five ranked classes representing (1) sand, (2) white-water, (3) mixed substrate, (4) deep consolidated rocky substrate and intertidal area, and (5) shallow consolidated rocky substrate. The output raster was then fuzzified using a large function.

Other factors included in the decision-support system were depth and exclusion areas. Maximum depth measurements were collected in-situ ( $n = 172$ ). The points were interpolated and create a digital elevation model (DEM). The DEM was then fuzzified using a Gaussian response membership. Previously established seeding plot waypoints were buffered to prevent overlap between seeded areas. This established an exclusion layer which was then rasterized and fuzzified using a linear membership.

All membership output raster's where overlaid using the “*And fuzzy overlay*” function to create the MCDSS. Results indicated that there was a significant correlation ( $r = 0.494$ ,  $p < 0.001$ ,  $n = 109$ ) between visual site-suitability assessment in-situ and the predicted likelihood of suitable seeding sites from the MCFDSS (Figure 1). The results from this research illustrate the value of using these systems in the planning and implementation phases of operations such as abalone ranching.



**Figure 1: Ground-Truthing of the MCFDSS showing the correlation between visual assessments and the predicted likelihood value.**

## **TRACING AQUACULTURE WASTE ACROSS A PRODUCTION CYCLE USING FATTY ACIDS AND STABLE ISOTOPES**

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The increase in global seafood demand has put pressure on fish oil and meal supplies, resulting in alternative sources, such as terrestrial based crops and products to produce aquaculture feed for finfish production. This dietary shift has resulted in creating terrestrial biomarkers, unique to the marine environment. These biomarkers can be used to identify and trace aquaculture waste, in the forms of excess feed and fish fecal material, into the marine environment and associated food webs. Norway is one of the largest producers of salmonid aquaculture in the world, producing 1.3 million tons annually. Expansion of the industry has involved the relocation of farms from protected fjords to coastal current rich areas. There is little knowledge on the impact aquaculture waste is having on the benthic environment and associated fauna within these dynamic coastal ecosystems. This study uses fatty acids and stable isotopes, to detect changes in biochemical composition of sediment along a transect from two salmon farms, and compare with reference locations across a production cycle. Fatty acids, associated with terrestrial origins, were found in higher concentrations within 200m of the farms, as well as displaying an increase across the production cycle. Shifting fatty acid profiles around aquaculture cages and the enrichment of benthic sediments could provide an additional trophic subsidy to organisms foraging in these coastal habitats, thus modifying the functioning of coastal benthic ecosystems where organic waste from salmonid aquaculture accumulates. In addition, we characterised the fatty acid and stable isotope ratios of key benthic fauna found under and adjacent to salmon farms to determine if they are consuming aquaculture waste.

## ESTIMATES OF ADDITIVE AND NON-ADDITIVE GENETIC EFFECTS FOR HARVEST BODY WEIGHT OF A COMPLETE DIALLEL CROSS OF THREE NILE TILAPIA (*Oreochromis niloticus*) STRAINS

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The aim of this study was to quantify the magnitude of additive and non additive genetic effects on harvest body weight (HBW) variation of different strains and strain combinations produced in a complete diallel cross of 3 Nile tilapia strains collected from different Ethiopian Rift Valley lakes. For this purpose, HBW of 2,421 fish from 81 full-sib families reared in two production systems were recorded and analysed. The results showed that the mean HBW of the fish reared in high input production system, Hi-P, (114 g) was 13% higher than in low input production system, Lo-P, (101 g). The results also revealed that there were substantial additive and non-additive genetic variations with moderate heritability estimates for HBW of *Oreochromis niloticus* in both production systems.

Re-ranking due to additive & non-additive genetic performances, of the tested strains & strain combinations in the two production systems, did occur, indicating the presence of genotype by environment interaction (GxE). However, the high genetic correlation between HBW recorded in the two production systems (0.82), implies only a low level of GxE. In conclusion, the substantial additive and non-additive genetic variations among the tested *O. niloticus* strains & strain combinations can be utilized to improve total genetic performance of the fish of both pure & hybrid lines in one of the production systems; preferably in the high input production system.

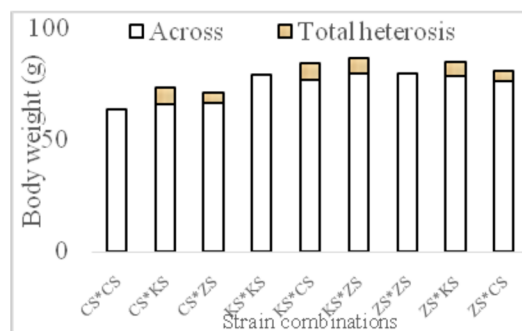


Figure 1. LSM of HBW of the 9 strain combinations of *O. niloticus* reared in the two production system

## REALIZED GENETIC GAIN FOR HARVEST BODY WEIGHT IN THE F<sub>2</sub> GENERATION OF A NILE TILAPIA BREEDING PROGRAM

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The aim of this study was to quantify the realized and predicted genetic gains of five months old Nile tilapia, *Oreochromis niloticus*, after one generation of selection for increasing body weight. The founder populations were collected from three Ethiopian Rift valley lakes, Lake Chamo, Lake Koka and Lake Ziway. Factorial mating design was applied in both generations. Body weight of 4,619 fish reared in different production environments were recorded for both generations. General leaner model analysis indicated that the mean harvest body weight for male and female fish increased from 109.5 g to 141.1g and from 37.3 g to 42.1 g, respectively. In average, the mean harvest body weight increased from 84.0 g to 109.1g.

The coefficient of variation for harvest body weight indicated the presence of large variation within generation. It was noted that the coefficient of variance for harvest body weight of the second generation for both sexes were relatively small compared to the coefficient of variance for harvest body weight of the first generation. The results also showed that the presence of moderate additive genetic variance for harvest body weight in both generations, resulted in moderate heritability estimates (0.20-0.34), with optimum realized genetic gain of 13.3% and predicted genetic gain of 10.0%. We conclude that optimum genetic gain was obtained in F<sub>2</sub> generation in which the genetically improved Nile tilapia (GINT) has better growth performance than the founder Nile tilapia strains.

Table 1 Predicted breeding values (BV) and least square means with their standard errors, predicted ( $\Delta$ PG) and realized ( $\Delta$ RG) genetic gains with their percentage (%) contribution for HBW of *O. niloticus* reared in different production environments

| Generation | BV       | LSM  | Genetic gain |              |             |              |
|------------|----------|------|--------------|--------------|-------------|--------------|
|            |          |      | $\Delta$ PG  | $\Delta$ PG% | $\Delta$ RG | $\Delta$ RG% |
| G1         | 0.24±0.8 | 78.2 |              |              |             |              |
| G2         | 8.01±0.1 | 88.6 | 7.8          | 10.0         | 10.4        | 13.3         |



## THE EFFECT OF SOYA AND CRYSTALLINE ISOFLAVONES ON THE GROWTH AND REPRODUCTIVE DEVELOPMENT OF FARMED ABALONE *Haliotis midae*

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The inclusion of soya as a dietary protein source in the formulated feed, Abfeed® S34 (Marifeed Pty (Ltd), Hermanus) for farmed abalone, *Haliotis midae* has resulted in larger gonads during reproductive seasons compared to the gonads of abalone fed a kelp-only diet or formulated feed that included fishmeal as the only main protein source. However, the addition of soya not only resulted in larger gonads, but also resulted in fastest growth and highest overall meat yield. It was suggested that the phytoestrogens present in soya were responsible for accelerated reproduction. However, the inclusion of crystalline isoflavone in a fishmeal-only based diet failed to verify the effects seen in abalone that had been fed soya diets. Abalone growth and reproductive development was found to be dose dependent on soya inclusion rates and this was dependent on time and sex of the abalone. In addition, female and male gonad histological assessments were done to determine the effect of graded levels of soya and crystalline isoflavones on gametogenesis. These findings will be presented.

## TLR22 STRUCTURE AND EXPRESSION CHARACTERISTIC OF BARBEL CHUB, *squaliobarbus curriculus* PROVIDES INSIGHTS INTO ANTIVIRAL IMMUNITY AGAINST INFECTION WITH GRASS CARP REOVIRUS

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Grass carp reovirus (GCRV) is the most virulent agent to Grass carp, *Ctenopharyngodon idella*, and causes a severe infectious disease called hemorrhagic disease of grass carp. Generally, barbel chub,

*Squaliobarbus curriculus*, a genetically closely related species to grass carp, exhibits significant resistance against GCRV infection compared to grass carp. To investigate whether the Toll-like receptor 22 (*tlr22*) has got a vital role against the GCRV infection, the full cDNA sequence of *tlr22* from barbel chub (*Sctlr22*) was cloned by RACE-PCR, and the structure and expression feature were studied.

The complete cDNA sequence of *Sctlr22* has a size of 3504 bp, encoding for 960 amino acid residues. *Sctlr22* possesses typical structural features of the *tlrs* family, including 19 leucine rich repeats (LRRs), a transmembrane (TM) and a Toll/interleukin-1 receptor (TIR) domain. Phylogenetic analysis revealed that barbel chub Tlr22 was clustered together with the Tlr22 of grass carp (*CiTlr22*). Structurally, barbel chub Tlr22 have two different structure in LRRs domain and TIR domain with grass carp (Susceptible to GCRV), but was similar to that of *Danio rerio* and *Cyprinus carpio* (Resistance to GCRV).

Quantitative RT-PCR analysis has shown that *Sctlr22* is prominently expressed in immune relevant tissues such as head kidney and spleen. After GCRV infection, *Sctlr22* expression level was up-regulated in four tested tissues and the highest expression of *Sctlr22* appeared fast and higher than *Citlr22*. The *interferon-β* (*ifn-β*) expression level in CIK cells over-expressing fused cDNA encoding the LRR domain of *Sctlr22* to the transmembrane and TIR domain of *Citlr22* was significantly higher than that cells overexpressing *Citlr22* after GCRV infection. The virus titer was significantly reduced compared to *Citlr22* over-expressing cells. These results suggested that *Sctlr22* seems to play a vital role in the immune response against GCRV.

## DISTRIBUTION OF VIRULENT *Aeromonas hydrophila* IN CHANNEL CATFISH TISSUES FOLLOWING WATERBORNE EXPOSURE

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In freshwater aquaculture, *A. hydrophila* was historically considered an opportunistic pathogen associated with secondary bacterial infections. After a 2009 outbreak of motile *Aeromonas* septicemia (MAS) in farmed catfish in West Alabama and East Mississippi, a virulent *A. hydrophila* (vAh) was isolated and determined to be responsible for the MAS outbreak. Tens of millions of pounds of market-sized catfish have been lost due to vAh. The vAh has emerged as a primary pathogen associated with catfish in the United States and carp aquaculture in China. In order to evaluate MAS disease progress and find effective methods to prevent/control vAh, we developed a reproducible waterborne challenge model recently. Although progress has been made in understanding vAh infection in catfish using this challenge model, it is still unknown when vAh gets access into fish from water, where the bacterium is distributed in fish tissues and how infection is progressed. This study evaluated the distribution of vAh in channel catfish tissues using real-time polymerase chain reaction (qPCR) following waterborne exposure with vAh.

Channel catfish were distributed in nine 57-L aquaria with 15 fish per tank to receive three treatments: 1) challenge with vAh at  $2 \times 10^7$  CFU/mL of water for determination of fish mortality, 2) challenge with vAh at  $2 \times 10^7$  CFU/mL of water for collection of fish tissues, and 3) mock challenge using sterile TSB as negative controls. After fish were anesthetized with MS-222 and adipose fin was clipped, fish were transferred into 57-L tanks filled with 15 L of water. For vAh challenge, 100 mL of vAh cell suspension was added to each tank resulting in  $2 \times 10^7$  CFU/mL of water. Following 1 h exposure, water flow was resumed. Fish mortality was monitored and recorded daily for 1 week post challenge. A total of 6 fish were randomly sampled among the 60 fish in treatment 2 at 1, 2, 4, 8, 24 and 48 h post vAh challenge. MS-222 euthanized fish were sampled following tissues for vAh quantification: blood, adipose fin near clipped site, gill, skin on the left side of lateral line just above pelvic fin, brain, intestine, liver, spleen and trunk kidney. Genomic DNA of fish blood and tissues was extracted using the DNeasy Blood & Tissue kit (Qiagen). A qPCR method was used to quantify cells of vAh in fish tissues.

Challenge with vAh resulted in 77.8% mortality and most mortality (about 91%) occurred within 48 h post challenge with mean-day to death of 1.5 days. Dead fish showed typical clinical signs of reddened fins, external/internal septicemia and hemorrhage. All dead fish sampled for confirmation were positive for the presence of vAh in liver tissue. At 2 h post challenge, vAh (genomic DNA copies or genome equivalents) was detected in all external and internal tissues sampled. Gill had the highest vAh cells at 1 h post challenge. Spleen harbored the most vAh cells among internal organs at 4 h post challenge. The tissues/organs with most vAh cells detected at 8 h post challenge were adipose fin, blood, intestine, kidney and skin while liver showed the highest vAh cells at 24 h post challenge. These results suggest that vAh was able to rapidly proliferate and spread, following wound infection, through the fish blood circulation system and cause mortality within 8 to 24 h.

## THE GENOME OF AMUR IDE (*Leuciscus waleckii*) PROVIDES INSIGHT INTO ADAPTIVE EVOLUTION IN RESPONSE TO EXTREME ALKALINITY

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The Amur ide (*Leuciscus waleckii*) is a cyprinid fish that is widely distributed in Northeast Asia. The Lake Dali Nur population inhabits one of the most extreme aquatic environments on Earth, with an alkalinity up to 50 mmol/L (pH 9.6), thus providing an exceptional model with which to characterize the mechanisms of genomic evolution underlying adaptation to extreme environments.

Here, we developed the reference genome assembly for *L. waleckii* from Lake Dali Nur. Intriguingly, we identified unusual expanded long terminal repeats (LTRs) with higher nucleotide substitution rates than in many other teleosts, suggesting their more recent insertion into the *L. waleckii* genome. We also identified expansions in genes encoding egg coat proteins and natriuretic peptide receptors, possibly underlying the adaptation to extreme environmental stress. We further sequenced the genomes of 10 additional individuals from freshwater and 18 from Lake Dali Nur populations, and we detected a total of 7.6 million SNPs from both populations. In a genome scan and comparison of these two populations, we identified a set of genomic regions under selective sweeps that harbour genes involved in ion homeostasis, acid-base regulation, unfolded protein response, reactive oxygen species elimination, and urea excretion. Our findings provide comprehensive insight into the genomic mechanisms of teleost fish that underlie their adaptation to extreme alkaline environments.

## Yalelo Limited

Yalelo Limited is one of Africa's leading aquaculture firms. Our name, Yalelo, means "Today's" in most of Zambia's local languages. This name communicates our two key competitive advantages in the fisheries sector: 1) we are "Today's" Zambian company with fresh ideas and technology and 2) we have "Today's" fresh fish, straight from Lake Kariba – not shipped from Asia.

Across the continent, rising incomes are driving an increased demand for fish, while overfishing is decreasing the amount of wild catch available. Zambia, in particular, has experienced devastating over-fishing during the last decade. Tilapia fish was once caught locally in abundance, but now most fish in urban markets is imported frozen. However, given a fresher, higher-quality alternative, consumers will switch to a local brand.

We breed and grow our tilapia in Siavonga, on the shores of Lake Kariba. Upon reaching market size of 450 grams we distribute our fish through eight wholesale warehouses and over eighty branded franchise retail shops within Zambia and Southern Africa.

## THE PROTECTIVE EFFECT OF HUMIC SUBSTANCES AGAINST FISH PATHOGENS

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Humic substances are organic, pigmented compounds that are common in terrestrial and aquatic habitats. In the current study, four different approaches were used to examine the possible health benefits of humic substances in fish:

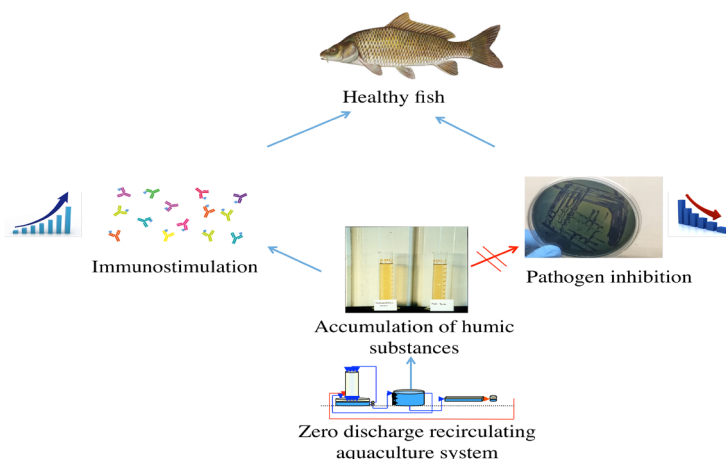
(1) Common carp (*Cyprinus carpio*) were exposed to humic-rich water and sludge from a recirculating aquaculture system (RAS) as well to a synthetic humic acid and a humic-rich extract from Leonardite. Carps exposed to these compounds through culture water and feed and challenged with the bacterial pathogen *Aeromonas salmonicida*, exhibited significant reduced infection rates. In the control treatment 46.8% of the fish were infected while exposure of the fish to (a) RAS culture water and sludge, (b) a synthetic humic acid and (c) an extract of Leonardite, reduced the infection rates to 14.9%, 17.0% and 18.8% respectively.

(2) Exposure of *Gyrodactylus turnbulli*- infected guppy (*Poecilia reticulata*), to RAS culture water and to the synthetic humic acid, significantly reduced the infection rate of guppy with this ectoparasite. While in the control treatment 52% of the fish were infected, in the RAS and humic acid treatments, infection rates were reduced to 17.2% and 25.3% respectively.

(3) Addition of above mentioned three different sources of humic substances to liquid cultures of *Aeromonas salmonicida* resulted in a significant growth reduction of the pathogen, ranging from approximately 40 to 60% of the growth of untreated bacteria.

(4) Common carp immunized against bovine serum albumin and reared in: (a) culture water from the RAS and fed with feed supplemented with dry sludge from the RAS and (b) synthetic humic acid-supplemented culture water and feed, displayed significant elevated antibody titers (log2) of 13.0 and 12.8 respectively, as compared to a titer of 10.0 in vaccinated carps that were not exposed to humic

**Figure 1. Protective effects of humic substances against pathogenic infection in fish as demonstrated in this study**



## CALRETICULIN FROM THE MUD CRAB, *Scylla paramamosain*: cDNA CLONING, PROFILES UNDER LOW TEMPERATURE AND SALINITY STRESS AND ITS RELATIONSHIP WITH $\text{Ca}^{2+}$ CONCENTRATION

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Calreticulin (CRT) is an important molecular chaperon crucial to survival of organisms under adverse conditions. In this study, cDNA cloning and the roles of CRT in the mud crab *Scylla paramamosain*, a warm-water species, when subjected to low temperature stress was investigated. The results showed that the full-length cDNA (*SpCRT*) was 1680 bp, and its open reading frame of 1211 bp was isolated and characterized. The expressions of *SpCRT* detected by real-time quantitative PCR (qRT-PCR) in various tissues of the crab were also measured and the highest expression was found in the hepatopancreas. When 28 °C acclimated sub-adult *S. paramamosain* subjected to lower temperatures of 10, 15, 20 and 25 °C, the profiles of *SpCRT* gene were detected in the hepatopancreas, chela muscle and gills. It showed that the expression levels of *SpCRT* mRNA in these tissues were significantly higher in the crabs exposed to 10 °C as compared to other 3 temperatures, and for those crabs exposed to 15 °C, the expressions were also significantly higher than those exposed to 20 °C and 25 °C. The high expression levels of *SpCRT* mRNA in crabs exposed to lower temperatures of 10 °C and 15 °C indicated that CRT was involved in cold adaptation, probably through facilitating protein folding. When low temperature further combined with both high and low salinity stress, it demonstrated that at a same low temperature (10 °C or 15 °C), the expression of *SpCRT* mRNA at low salinity of 10 was mostly significantly higher than that at high salinity of 35, suggesting that when subjected to low temperature stress, low salinity may do more harm to the crabs than high salinity. It was also shown that at 10 °C,  $\text{Ca}^{2+}$  concentration increased in the hepatopancreas and a further *in vitro* experiment showed that the expression of *SpCRT* mRNA increased with the added  $\text{Ca}^{2+}$  concentration. These results together imply that  $\text{Ca}^{2+}$  probably plays a major role in low temperature signaling, which could induce expression of genes related to cold adaption, including CRT.



## EFFECTS OF SUBSTITUTING FISH OIL WITH MIXTURES OF VEGETABLE OILS ON GROWTH PERFORMANCE, FATTY ACIDS AND FLESH QUALITY OF RAINBOW TROUT

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The aim of this study was to determine the effects of dietary replacement of fish oil with a mixture of vegetable oils on the fatty acid composition of the fillet and liver and the flesh quality traits of rainbow trout (*Oncorhynchus mykiss*) after a 70-day feeding. Four iso-nitrogenous (approx. 51% crude protein) and iso-lipidic (approx. 14% crude lipid) experimental diets were formulated. Fish oil (FO) was the primary lipid source in the control diet. In the other three dietary treatments, fish oil was replaced by 100% (LO30/SO35/SFO35) and 70% (FO30/LO35/SO35 or FO30/SO35/SFO35) sesame oil (SO), linseed oil (LO), or sunflower oil (SFO). Triplicate groups of 40 rainbow trout (~46 g) held under similar culture conditions were hand-fed daily to apparent satiation for the duration of the study. At the end of the feeding trials, growth performance, hepatosomatic index and viscerosomatic index were not influenced by experimental diets ( $P > 0.05$ ). Fillet proximate composition of fish was influenced by dietary treatments ( $P < 0.05$ ): the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels were highest in fish fed the control diet ( $P < 0.05$ ); and EPA and DHA levels in fish fed the FO30/LO35/SO35 diet were closest to the control diet ( $P < 0.05$ ). Moreover, fish fed FO30/LO35/SO35 diet had significantly higher level of 18:3n-3 than those in the other fish groups ( $P < 0.05$ ). In contrast, fish fed the diet containing 100% plant oils (LO30/SO35/SFO35) had the highest level of total n-6 fatty acids in the fillet and liver. However, docosahexaenoic acid (DHA) levels in liver were higher than those in the fillet of all dietary groups. In a 12-day refrigerated storage at 1°C the thiobarbituric acid (TBA), trimethylamin nitrogen (TMA-N) and pH values gradually increased in all dietary groups ( $P < 0.05$ ). However, the values of these chemical indicators of spoilage, TBA, TMA-N and pH, were within the limit of acceptability for human consumption. The results suggested that 70% (FO30/LO35/SO35 or FO30/SO35/SFO35) substitution of fish oil with a mixture of sesame, linseed or sunflower oils could be used in rainbow trout diets.

## HETEROSIS AND HYBRIDIZATION ON *HALIOTIS*: EXPERIMENTAL EVIDENCE AND ITS APPLICATION ON CHINESE ABALONE INDUSTRY

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Abalone aquaculture is developing very rapidly in the last twenty years in China. The annual production and values of farmed abalone reached to 130,000 tons and 2 billion US Dollars in 2014, respectively. Following with the large-scale cultivation of abalone, a series of problems including germplasm degeneration, frequentation of disease and obviously decreased growth rate of farmed abalone have been emerged. Faced with these problems, the long-term genetic improvement program for farmed abalone has been supported by government and initiated since 2001. Growth and resistance to stress/disease has been identified as traits of interest for genetic improvement on *Haliotis diversicolor* and *H. discus hannai*. Different abalone species or geographical populations were collected or introduced to establish the abundant germplasm banks, while genetic background were analyzed by molecular markers. Pedigree based inbred families/lines were built and divergent selection were conducted for several generations. Then, interspecies and intraspecies hybridization were conducted by complete diallel cross designs, while the traits of hybrids and backcrosses were tested and evaluated on the different farming model and places. Some specific hybrids show positive heterosis for growth and disease/thermal resistance abilities. Until now, three hybrids were conferred for “new species certificate” by Ministry of Agriculture and now are large-scale cultured in China. Furthermore, the researches on the application of molecular marker and omics approaches in parentage analysis, population genetics and high-density genetic map were also performed.

## PATHOPHYSIOLOGICAL ASPECTS OF THE INNATE IMMUNE RESPONSE OF *Piaractus mesopotamicus* ALLOXAN-DIABETICS CHALLENGED WITH *Aeromonas hydrophila*

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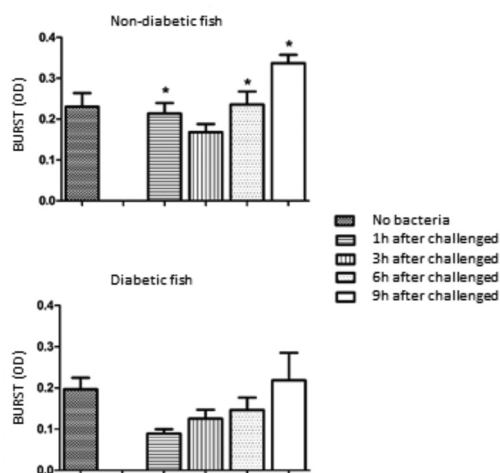
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Pacu (*Piaractus mesopotamicus*) as a model for the study of inflammation has advantages over other animal models, especially in the study of inflammatory process. The present study aimed to elucidate the interactions of acute inflammation induced by *Aeromonas hydrophila* inactivated in diabetics' pacu. For this, 350 pacus ( $\pm 110$ g) were distributed into 2 groups, diabetic and non-diabetic. Each group was distributed into two new groups, control (injected with saline) and inoculated with *A. hydrophila*. The last group was evaluated at 1, 3, 6 and 9 hours after challenge.

After euthanasia with benzocaine, samples were collected for hematological variables, serum biochemistry, glycaemia, respiratory blood leukocyte activity, lysozyme concentration, serum lytic activity, bacterial agglutination activity and histopathology. It was observed that hematocrit, hemoglobin, number of erythrocytes, CHCM, HCM, albumin, globulin and respiratory burst decreased in diabetic fish probably related to microcytic hypochromic anemia attributed to iron consumption by bacteria and compromised immune response.

However, these fish also had higher levels of leukocytes, granulocytes and monocytes, probably related to the exacerbated increase of the leukocyte adhesion in the vascular endothelium, impairing the immune response. The decrease in triglycerides and cholesterol was related to the increase in energy demand due to the absence of insulin. It was also found that renal and hepatic functions were not altered by the use of alloxan. There were no differences in plasma levels of lysozyme, agglutination and thyroxine. The progressive decrease of triiodothyronine was related to an increase of lipid metabolism in order to supply energy to the immune system to control the infection.

We conclude that alloxan was effective in inducing diabetes with preservation of renal and hepatic function. The results indicate that the process of induced diabetes and inflammation present adequate characteristics to make this fish a model of study of this disease.



## ALLOXAN-INDUCED DIABETES IN *Piaractus mesopotamicus* AND *Oreochromis niloticus*

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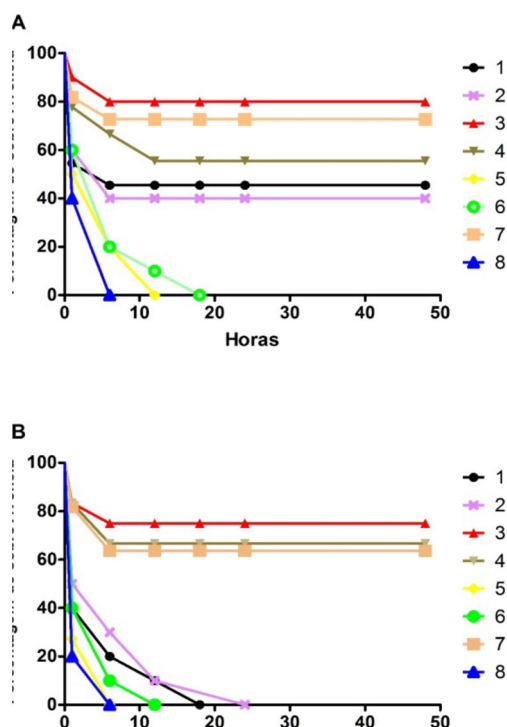
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There are limitations in experimental animal models for the study of diabetes. The proposal of new models implies the standardization of techniques and protocols necessary for its development. The objective of this study was to establish protocols for the induction of diabetes in pacus (*Piaractus mesopotamicus*) and Nile tilapia (*Oreochromis niloticus*) by the method of pharmacological exclusion of insulin production by treatment with alloxan.

For this, 8 protocols were tested in each species based on mammalian studies and previous trials performed for this study. The variables evaluated were injection route, dose, concentration and volume of the vehicle and use of anesthesia. Intravenous injection of 150 mg / kg of alloxan diluted in 2 mL of citrate buffer 0.01M pH 4 was found to produce the lowest mortality and the highest amount of alloxan-diabetic animals in pacus and Nile tilapia. It was also verified that there was necrosis of the pancreatic cells, responsible for the synthesis of insulin.

Pacus showed lower mortality and higher rates of diabetes-induced animals than Nile tilapia. It was concluded that this protocol is the most appropriate for studies of diabetes in these species.



Acknowledgements: Grants # 2015/14289-8 and 2016/18345-2, São Paulo Research Foundation (FAPESP) and #446917/2014-1 CNPq.

## **SAPROLEGNIOSIS IN NILE TILAPIA *Oreochromis niloticus*: IDENTIFICATION, MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF TWO NOVEL PATHOGENIC *Saprolegnia* SPP. STRAINS**

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Saprolegniosis is a fungal infection that occurs in winter, resulting in mass mortalities and decreases in egg hatchability with subsequent huge economic losses in Egyptian tilapia fish farms. In Egypt, Nile tilapia fish are considered the most commonly cultured fish species; therefore, Saprolegniosis represents a serious problem for the Egyptian tilapia aquaculture industry. *Saprolegnia* spp. are usually implicated as the etiological agents, but their identification is sometimes troublesome and confusing. In this study, two *Saprolegnia* strains (ManS22 and ManS33) were isolated from Nile tilapia (*Oreochromis niloticus*) suffering from saprolegniosis. Both isolates were characterized morphologically and from internal transcribed spacer (ITS) sequence data. Additionally, both strains, ManS22 and ManS33 were tested for pathogenicity, and they were highly pathogenic and caused cumulative mortalities of 88.9% and 95.6%, respectively. Initially, the two strains were identified, by morphology of sexual and asexual stages, as members of the genus *Saprolegnia*. For more definitive identification and characterization, the ITS region of the ribosomal RNA genes was amplified and sequenced, and sequences were compared with other known sequences in GenBank. A phylogenetic tree constructed using the neighbor-joining method revealed that the two strains fell into two clusters within the species *Saprolegnia parasitica*. Cluster 1 included the ManS33 strain and cluster 2 the ManS22 strain. Cluster 1 grouped the ManS33 strain with other *S. parasitica* strains and shared 97–99% sequence similarity. Cluster 2 contained only the ManS22 strain and shared 93–94% similarity to several reference sequences of *S. parasitica* strains. Therefore, our findings suggest that ManS22 represents a newly described strain of *S. parasitica*. Since Malachite green uses has been banned in fish farms worldwide owing to its carcinogenic effects, no control measures have been efficient to protect against saprolegniosis especially after hatching. Recently, some researchers reported effector proteins that are important in the oomycetes pathogenicity. Therefore, molecular characterization of different *saprolegnia* strains will help in identifying the effector proteins that could be as vaccine candidates to combat saprolegniosis.

## EFFECTS OF CHLORPYRIFOS EXPOSURE ON HEMATOLOGICAL PARAMETERS, ANTIOXIDANT STATUS, AND IMMUNE GENE EXPRESSION IN NILE TILAPIA *Oreochromis niloticus*

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Chlorpyrifos (CPF) is considered one of the most common insecticides in freshwater ecosystems, and has been detected in agricultural and fishery products. This study focused on potential toxic effect of CPF on hematological responses, antioxidant activity, and immune gene expression in Nile tilapia (*Oreochromis niloticus*). Nile tilapia fish (*Oreochromis niloticus*) was exposed to CPF at 15  $\mu\text{g/l}$  (1/10  $\text{LC}_{50}$ , group CPF1), or 75  $\mu\text{g/l}$  (1/2  $\text{LC}_{50}$ , group CPF2) for 14 days, followed by 15 days recovery. Different endpoints were used to determine effects of CPF on fish health: hematological parameters (RBCs, Hb, PCV, MCV, MCH, total and differential leukocyte counts); antioxidant levels in liver and gills (malondialdehyde /MDA/, catalase /CAT/, glutathione /GSH/, glutathione S-transferase /GST/, and superoxide dismutase /SOD/); innate immune parameters (bactericidal activity, lysozyme activity, and respiratory burst activity); and expression levels of cytokine genes at mRNA level in anterior kidney and spleen (IL-1 $\beta$ , TGF- $\beta$ , TNF- $\alpha$  and IL-8). Significant changes were detected in all hematological parameters measured at days 3, 7, and 14 in CPF exposed groups. Antioxidant enzyme activity in liver and gills showed significant increase of MDA and GSH, and significant decrease in CAT, GST, and SOD in CPF treated groups. Bactericidal activity was significantly decreased, but respiratory burst was significantly increased in CPF2 group. The cytokine expression levels showed complex changes in expression patterns. In kidney, cytokine IL-8 was significantly increased on day 1, then decreased at day 3 in both CPF groups. IL-1 $\beta$  expression level was increased significantly on day 1 in the CPF1 group. TNF $\alpha$  was downregulated in both CPF groups on day 3 followed by upregulation in group CPF1 on day 7. All gene expression levels in spleen were upregulated on day 7 post exposure. Following recovery period (15 days after stopping of the CPF treatment), antioxidant levels decreased to control levels, bactericidal activity improved, and cytokine expression levels were downregulated compared to their levels before recovery. The CPF exposure has adversely affected hematological parameters, induced oxidative stress response, changed innate immune parameters, and cytokine expression levels in tilapia. These findings provided important insights about the toxic effects of CPF on fish and show potential to be used as biomarkers in further toxicological evaluation studies.

## IDENTIFICATION AND GENE EXPRESSION OF INTERFERON REGULATORY FACTORS IN *Megalobrama amblycephala*

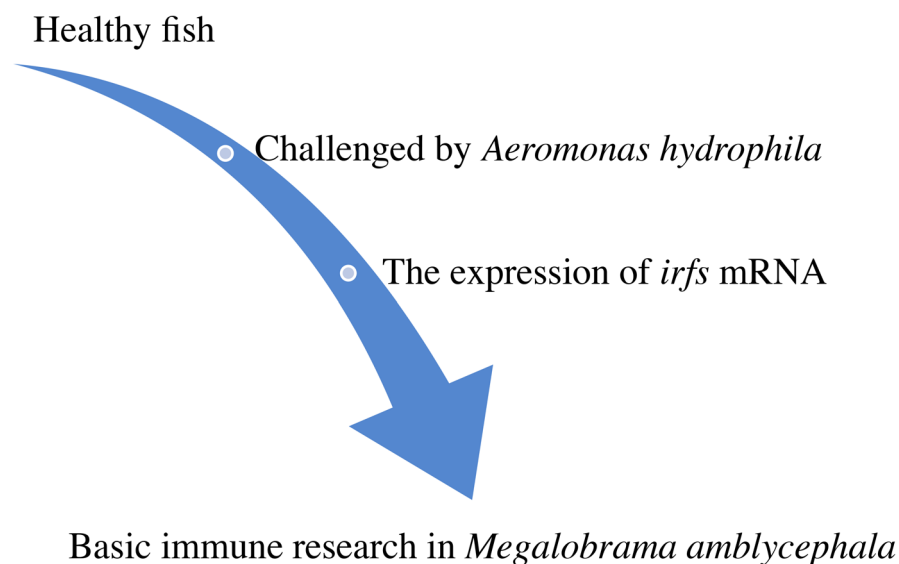
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The family of interferon regulatory factors, which includes nine mammalian members (*irf1–irf9*), acts as transcription factors for interferons and exerts regulatory functions in the immune system and in oncogenesis. And irfs are known to be critical in regulating a spectrum of functional and developmental processes in lymphomyeloid cell lineages through direct binding to interferon stimulatory response elements in target gene promoters of immune or immune-related genes. In the present study, this family members (*irf1*, *irf2*, *irf4a*, *irf4b*, *irf6*, *irf7*, *irf8*, *irf9*, *irf10*) have been identified and characterized in blunt snout bream all sharing homology in a well conserved DNA binding domain (DBD) covering the first 115-120 amino acids in the N-terminus, with the characteristic motif containing a series of tryptophan residues. Phylogenetic analyses confirmed the all irfs belong to four irfs sub-families and revealed close relationships with cyprinid orthologs. In healthy fish, *irfs* mRNA were most abundant in a range of nine tissues, especially in the liver spleen and kidney, where the expression of *irf1* mRNA was highest. The expression of *irfs* mRNA displayed an obvious dose-dependent manner following *Aeromonas hydrophila* challenge, *irf4a*, *irf4b* and *irf10* were up-regulated in the detected tissues, while *irf1* was down-regulation in intestine and gills, *irf2*, *irf6*, *irf8* and *irf9* were down-regulated in liver. However, the expression of *irf7* mRNA was down-regulated in the detected tissues. Overall, these data may be indicating that these irfs more likely involved in antibacterial immune response.





## VARIATIONS OF IMMUNE PARAMETERS IN THE LINED SEAHORSE *Hippocampus erectus* AFTER INFECTION WITH ENTERITIS PATHOGEN OF *Vibrio parahaemolyticus*

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Enteritis has been increasingly recognized as one of the major obstacles for the lined seahorse *Hippocampus erectus* mass culture success. In the present study, the intestinal bacteria strains of the lined seahorse *H. erectus* suffered from enteritis were isolated, then their pathogenicities were confirmed by artificial infection, and one pathogenic bacteria strain named DS3 was obtained. The median lethal dose ( $LD_{50}$ ) strain DS3 for 10 days, was determined. The seahorses with different infection levels of uninfected (control), early stage of infection (ESI) and late stage of infection (LSI) were respectively sampled at 0, 3, 6 and 9 days post infection, and 12 immune parameters in the plasma were analyzed. The strain DS3 identified with a biochemical test combined with a molecular method was *Vibrio parahaemolyticus*, and its  $LD_{50}$  for 10 days was  $1.3 \times 10^3$  cfu/fish. Six parameters including monocytes/leucocytes, leucocytes phagocytic rate, immunoglobulin M, interleukin-2, interferon- $\alpha$  and lysozyme exhibited a generally similar variation trend: highest in the control, second in ESI and lowest in LSI during the entire experimental period. In view of the infection level of *V. parahaemolyticus* to *H. erectus* is largely decided by the seahorse's own immune capacity, therefore, these immune parameters were high in the non- or slightly infected seahorses, and low in the severely infected individuals may be an indicator for immune level. The immune parameters may be reliable indicator for the juvenile and broodstock quality assessment. Moreover, clarification of the enteritis pathogen also provides guidance for targeted medicine choice for the lined seahorse.

## FINANCIING FOR THE ESTABLISHMENT OF SMALL-SCALE INTEGRATED FISH FARM IN BOSTWANA

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The financing of small scale fish farming in Botswana is currently non-existent, as fish farming is still virtually unknown. With fish farming being placed under Ministry of Wild Life & fisheries Departments, bears the syndrome of emerging markets, the absence of policy and legislation hinder decision making criterion and has slowed the growth pace of this industry. After ten years since inception, Discus Den Aquatics (Pty) Ltd (DDA) has barely traded, but however, with its limited resources, DDA embarked on a pilot project for breeding, rearing, processing and sale of fish. The project has been successful at breeding at polyculture level, the final stage would encompass cage 'hapas' system.

Research on genetic characteristics on local breeds rates, growth rates were carried out over a period of two years and recorded. Due to the high costs of design and construction, DDA was unable to complete its production plan and seeks assistance to the tune of ±1m. See summary of the capital costs below.

DDA is a 50 m x15m x3m pond reservoir belonging Botswana Railways, on a 5-year lease. The land area is 20 x 100m to accommodate the hatchery section, primary and secondary nurseries and grow out section, processing, storage, feed processing and sales. At full scale, the facility has potential to produce 40/60 tonnes of fish per year with a market value of approximately P1, 600,000 supported by an integrated vegetable section for feed supplements. DDA has, for the past 3 years, initiated developments with limited resources.

Botswana is a desert country, but, has very high and good quality underground water that could supply aquaculture projects, complemented by excellent temperatures that could prove ample for tilapia (*oreochromis*) growth rates.

This project will mark a blueprint in Botswana's road to fish farming success.

| TABLE 1: CAPITAL COSTS         |  |     |              |                   |                      |
|--------------------------------|--|-----|--------------|-------------------|----------------------|
| ITEM                           |  | QTY | PRICE (Pula) | TOTAL             | US\$ (RATE 0.979)    |
| <b>HATCHERY</b>                |  |     |              |                   |                      |
| Design & Construction          |  | 1   | 90849.1      | 90849.1           | USD 8,894.13         |
| Equipment & Fittings           |  | 1   | 116332.85    | 116332.85         | USD 11,388.99        |
| <b>GROWOUTS</b>                |  |     |              |                   |                      |
| Equipment & Fittings           |  | 1   | 26725        | 26725             | USD 2,616.38         |
| <b>PROCESSING</b>              |  |     |              |                   |                      |
| Equipment                      |  | 1   | 4575         | 4575              | USD 447.89           |
| <b>STORAGE</b>                 |  |     |              |                   |                      |
| Deep Freezers                  |  | 1   | 39599.8      | 39599.8           | USD 3,876.82         |
| Blast Freezers                 |  | 1   | 109536       | 109536            | USD 10,723.57        |
| <b>TRANSPORTATION</b>          |  |     |              |                   |                      |
| Pickup Truck                   |  | 1   | 231923.59    | 231923.59         | USD 22,705.32        |
| Refrigerated Trailer (1 tonne) |  | 1   | 394000       | 394000            | USD 38,572.60        |
| <b>TOTAL</b>                   |  |     |              | <b>1013541.34</b> | <b>USD 99,225.70</b> |

# ADDENDUM

## FEEDS AND FEEDING FOR SUCCESSFUL SHRIMP PRODUCTION

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The growth of global shrimp production has slowed in recent years as producers contend with emerging diseases, aging ponds, and price pressures. Differing farming strategies have been devised to improve financial sustainability of the industry in response to these challenges. In Ecuador, industry consolidation, more extensive culture systems, lowering of cost of production have been coupled with breeding and disease control strategies that rely on pathogen exposure and breeding for resistance. On the other end of the spectrum, smaller more intensive production strategies rely on high yields, higher financial inputs, biosecurity and fast growing SPF stocks. Managers are faced with difficult decisions based on very different farming models, often attempting to apply hybrid strategies with mixed results. One factor that unifies the successfully managed most profitable farms anywhere in the world is the efficient application of high quality feeds.

Feeds drive the culture system providing the primary source of health and nutrition for the target crop. They directly and indirectly affect water and substrate conditions that often determine harvest success. Formulation, manufacturing and feeding practices must be optimized to maximize profits in any given culture system. High quality aquafeeds must be carefully formulated to meet the nutrient demands of the shrimp. The nutrient density of the feeds may vary depending upon farming strategies but all feeds must meet digestible nutrient requirements of the shrimp in a given culture environment. New sources of high quality proteins and fats are providing more options for sustainable high quality feeds based on nutritionally complete formulations. In aquaculture systems, feeds are crucial for maximizing health and fitness of the target crop. Supplements and additives when properly applied can improve shrimp immune balance while helping to reduce opportunities for disease outbreaks. When properly balanced, micronutrients are a key component enabling these health benefits. Advances in feed production technology support higher energy efficiency and improved throughput while enabling better feed physical qualities. Manufacturing processes play a critical role in feed performance. Shrimp feeding behavior necessitates water stability, appropriate texture and sizing to maximize performance. It is, however, in the application of high quality feeds where one of the greatest opportunities for improving production performance lies. The most efficient shrimp farm managers across the aquaculture industry focus on precision feeding to maximize nutrient conversion into harvestable biomass while minimizing the waste which drives environmental deterioration. New technologies are pushing down FCRs by applying improved feed program software and even using hydrophones to measure feeding rates. As strategies for farm design, breeding, seed selection, pond preparation, stocking densities and disease control are selected and applied, the overarching importance of proper application of high quality feeds will remain a fundamental prerequisite to the profitability and sustainability of any commercial shrimp farming operation.

## **KIKKONET IN FISH FARMING - MARINE AND FRESHWATER**

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KikkoNet provides the best opportunity for less maintenance, better protection and safety for the fish in today's aquaculture.

KikkoNet is a high performance net constructed from PET (polyethylene terephthalate) monofilaments with a double twisted hexagonal mesh.

The PET monofilament is a single, hard and smooth plastic wire; the surface area is limited compared to multifilament wire making it difficult to deposit eggs between the fibres. Growth of biofouling is limited and fouling detaches relatively easily thus resulting in high dissolved oxygen levels in the water flowing into the cage.

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In 2014 – 2015 KikkoNet was installed in several submersible SeaStations moored off the coasts of Panama and Hawaii.

Since its introduction in 2010 hundreds of KikkoNet systems have been successfully installed in Scandinavia, the Shetland Islands, Australia and in the Americas, and continue today to protect the valuable live-stock of numerous fish farming companies without ever having been replaced.

## **SETTING A NATIONAL AQUACULTURE PRODUCTION TARGET AND MISSING IT MIGHTILY: REVIEWING AND EXPLAINING CAUSES FOR THE CASE OF GHANA**

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The past 10 years saw a frenzy of national initiatives aimed at dramatically increasing aquaculture production in sub-Saharan Africa; a region generally believed to be producing well under its potential and beset with some of the largest and persistent gaps between fish production and domestic consumption or requirement. In the face of declining catches from inland and marine sources, and a stagnant to sluggish growth in fish production over several decades, Ghana experienced a 40-60% deficit between domestic fish production and consumption over several decades. A growth in aquaculture (primarily Tilapia) production from < 20,000 MT yr<sup>-1</sup> in 2011 to 100,000 MT in 2016 (year later revised to 2018) became the central objective of the Ghana National Aquaculture Development Plan, published by the Fisheries Commission in 2012.

Although the Fisheries Commission and its development partners outlined a seemingly robust strategy to achieve the 100,000 MT target, growth of production has been hampered by many technical and macroeconomic constraints to the extent that it is yet unclear whether Ghana can reach even half of the originally set target in 2018. In this contribution, I use data from a study commissioned by the FAO on the social and economic performance of tilapia farming in Ghana and other African countries, along with a decade of research and observations in Ghana, to examine the myriad reasons for the failure to achieve the target. I also fit non-linear regression models to the historical production data to predict future fish production under various aquaculture growth scenarios.

Among other issues, high cost of commercial feeds exacerbated by an unstable currency and excessive reliance on imported inputs and cheaper foreign fish, extremely high cost of energy and unreliable electricity supply, high cost of borrowing, lack of a well-developed nursery sector, inadequate extension services and lack of a development-oriented domestic research agenda are examples of the macroeconomic growth constraints identified. The lack of adequate extension also manifests in improper pond construction, brood management, and stocking and feeding practices, and inaccurate and inconsistent record keeping. The ministry appears to have been trapped in problem identification rather than problem solving, leading to a little or no progress in removing obstacles that have been pointed out by too many exploratory studies.

In sub-Saharan Africa, the discrepancy between aquaculture development vision and reality is not unique to Ghana, and in different contexts, there does not appear to be a single, simple solution to the slow pace of development. The most promising avenue to accelerating aquaculture development, however, is in governments improving macroeconomic policies.

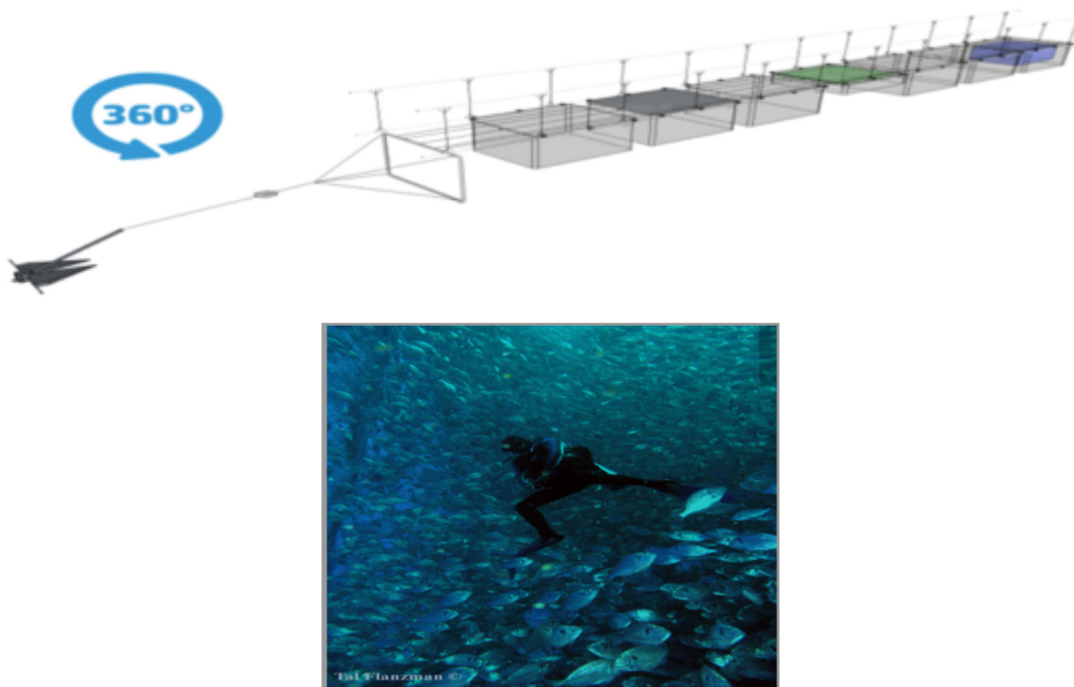
## TECHNOLOGICAL INNOVATION IN OFFSHORE FARMING - THE CASE STUDY OF GILIOCEAN TECHNOLOGY'S, NEW, 2000 TNY, SUBMERSIBLE OPEN OCEAN NETCAGES

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GiliOcean Technology, is the solution to the challenges that traditional fish farms face today. By moving fish offshore, back into their natural habitat where the oxygen level is ideal and they can feed on their natural microelements feed supplements, fish can grow faster and healthier, with negligible use of antibiotics. This leads to a premium quality of marine protein.

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*(Continued on next page)*

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## RECIRCULATING POND SYSTEMS: MODELS FOR SHRIMP FARMING IN AFRICA

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Limitations on the availability of land, water or both, and the desire for increased control over the production environment have led some commercial shrimp producers (stocking density 10-15/m<sup>2</sup>) in Latin America to develop recirculating pond systems. Although water exchange continues to be a common practice, it can result in sedimentation of water supply canals and ponds, poor biosecurity, poor pond water quality, difficulty regulating salinity, and difficulty establishing mature and stable microbial and algal communities. Water exchange also displaces the waste treatment function from inside the farm to the surrounding environment.

Properly designed treatment systems include basins for sedimentation and oxidation and one or more circulation pumps. Significant treatment occurs in drainage canals, operating as oxidation ditches. In the main reservoir, finger-like projections of embankment direct water in a serpentine flow, improving treatment capacity. All water introduced to the farm from outside should be treated in sedimentation ponds before adding to recirculated water.

At the farm level, the main benefits of recirculation are reduced sediment accumulation, reduced water pumping requirements, and improved biosecurity. Ponds can be managed to stock and harvest at different times, so carrying capacity can be “shared” among ponds in a block. A secondary benefit to recirculation is the development of “mature” water that can be effective as a method to control or limit EMS. The main costs of establishing a recirculating are associated with a reduction in productive area by 10-15% and the additional investment and construction costs.

At the pond level, the main benefit to recirculation is likely related to water movements. Relative to static ponds, water movement, even in hypereutrophic conditions, increases the supply rate of dissolved oxygen to cultured animals, including benthic-dwelling shrimp. Movement can be accomplished by mixing individual ponds, but it is more efficient and practical to apply energy to circulate water with one main recirculating pump station. To maximize the benefit of water movement, long and narrow ponds are best.

If maximum feeding rate (=carrying capacity) is restricted to 30-35 kg/ha per d, then processes internal to ponds are sufficient to maintain good water quality, and only a small reservoir (<10% of area) is needed. As the intensity of pond production increases, the proportion of the farm area in reservoir increases, up to 80% for intensive shrimp production. The configuration of most recirculating pond systems is based much more on practical experience than on application of rational waste treatment design approaches or a clear understanding of pond ecology. Nonetheless, these systems enable good crop production (10-15 t/ha) without widespread use of paddlewheel aeration.

## ECOLOGICAL RISK ASSESSMENT IN AQUACULTURE - A NEW APPROACH

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Global advancement of aquaculture is increasingly based on the farming of non-native species. This trend is set to continue as farmers search for new species that perform better under domestic conditions. Moreover, recirculating and closed farming systems, and the ability to control production conditions, has contributed to this phenomenon. The globalisation of species such as Atlantic Salmon, Rainbow Trout, Pacific Oysters, Mediterranean Mussels and Nile Tilapia is well-known, and there is little doubt that this list will grow. The unchecked introduction of new species has the potential to cause ecological harm through hybridisation, the introduction of novel diseases, habitat alteration and species displacement through predation or competitive behaviour.

The assessment of ecological risk has been applied widely as a predictive tool to aid decision making around non-native species. Conventionally, such assessments relied on the characterisation of risk by means of the relationship between probability of occurrence, severity of the potential impact, scope, and in some instances, permanence. In many methods, the risk is quantified by the allocation of justified numeric scores to probability, severity, scope and permanence. However, ignoring the effect of certainty (confidence) in this, as well as the potential for monitoring and mitigation, may under or overestimate the risk.

This paper shows that ecological risks can be characterized by allocating a weighted score to probability, severity, scope and permanence, as well as confidence, monitoring and mitigation. The sum of these result in a quantification in which a high score (max 100) represents a significant risk and a low score (min 7) represents a low risk.

The manifestation of an ecological risk is fully dependent on the pathway that facilitates occurrence. Notwithstanding generic impacts of aquaculture, the introduction of non-native species can only pose a risk through two pathways; these being escape and acting as a vector for disease. Given the full dependence of the risk on the pathway, this is the critical point of mitigation associated with the use of non-native species.

| Probability | High         |    |    |    | Moderate     |    |    |    | Low      |    |    |   | Extremely Low |   |   |   | Negligible |   |   |   |
|-------------|--------------|----|----|----|--------------|----|----|----|----------|----|----|---|---------------|---|---|---|------------|---|---|---|
|             | 20           | 19 | 18 | 17 | 16           | 15 | 14 | 13 | 12       | 11 | 10 | 9 | 8             | 7 | 6 | 5 | 4          | 3 | 2 | 1 |
| Severity    | Very high    |    |    |    | High         |    |    |    | Moderate |    |    |   | Low           |   |   |   | Negligible |   |   |   |
|             | 20           | 19 | 18 | 17 | 16           | 15 | 14 | 13 | 12       | 11 | 10 | 9 | 8             | 7 | 6 | 5 | 4          | 3 | 2 | 1 |
| Scope       | Extensive    |    |    |    | Regional     |    |    |    | Local    |    |    |   | Project based |   |   |   | Negligible |   |   |   |
|             | 15           | 14 | 13 |    | 12           | 11 | 10 |    | 9        | 8  | 7  |   | 6             | 5 | 4 |   | 3          | 2 | 1 |   |
| Permanence  | Permanent    |    |    |    | Long-lasting |    |    |    | Moderate |    |    |   | Temporary     |   |   |   | Short-term |   |   |   |
|             | 15           | 14 | 13 |    | 12           | 11 | 10 |    | 9        | 8  | 7  |   | 6             | 5 | 4 |   | 3          | 2 | 1 |   |
| Confidence  | Doubtful     |    |    |    | Low          |    |    |    | Moderate |    |    |   | High          |   |   |   | Very high  |   |   |   |
|             | 10           |    | 9  |    | 8            |    | 7  |    | 6        |    | 5  |   | 4             |   | 3 |   | 2          |   | 1 |   |
| Monitoring  | Zero         |    |    |    | Low          |    |    |    | Moderate |    |    |   | High          |   |   |   | Very high  |   |   |   |
|             | 10           |    | 9  |    | 8            |    | 7  |    | 6        |    | 5  |   | 4             |   | 3 |   | 2          |   | 1 |   |
| Mitigation  | Irreversible |    |    |    | Low          |    |    |    | Moderate |    |    |   | High          |   |   |   | Very high  |   |   |   |
|             | 10           |    | 9  |    | 8            |    | 7  |    | 6        |    | 5  |   | 4             |   | 3 |   | 2          |   | 1 |   |

## EVALUATING SALINITY EFFECT ON THE PERFORMANCE OF VARIOUS *Penaeus vannamei* FAMILIES

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Water Salinity has a profound effect on the food consumption, osmoregulation, energy demand, nutrient utilization, and molting and metabolic rate of shrimp. Optimal salinity range for the growth of penaeid shrimp in the juvenile stage is between 15 to 25 parts per thousand (ppt). However, as euryhaline species, shrimp could grow in brackish water which may be the only available water resource for growing shrimp in some inland aquaculture farms. For these farms, fast growing strain of shrimp in low salinity water would be highly desirable. To assess the effects of shrimp genetics and salinity on the performance of shrimp, 10 families of specific-pathogen-free *Penaeus vannamei* were tested in the cages in two pond with 2ppt and 32ppt water.

Sets of 10 cages (1.2m (L) X 1m (W) X 1.5m (H)) were placed in each of the two outdoor ponds (13m (L) X 13m (W) X 1.5m (D)). The two ponds were initially filled with seawater, and 200 SPF shrimp juveniles from each of the ten families were stocked in the cages of the two ponds. One pond was kept with the seawater (32ppt), and the other pond was gradually changed from seawater to freshwater (2ppt) per week. 45 shrimp of each of the families from the two ponds were sampled every two weeks for growth. At the end of the trials, final weight, survival, as well as tissue samples were sampled for RNA extraction, followed by cDNA construction, and consequently gene expression analysis. The familial difference in terms of various indexes was evaluated for genetic and environment interactions, as well as incorporated in potential family-based selection under low salinity condition.

Interestingly, all shrimp families grew faster in freshwater than in seawater during the first six weeks even including the acclimation period. The results also indicated that there were significant familial differences in terms of growth, survival, gene expressions of ATP synthase, Trypsinase, Na<sup>+</sup>-K<sup>+</sup>-ATPase and a Toll-like gene in various tissues. Two families demonstrated superior performance in both salinities as well as gene expressions in comparison with other families, which may qualify them as good candidates for breeding stock for selection in terms of growth traits regardless of the salinity. Among the other eight families, one family exhibited higher growth rate in the low salinity water than the regular seawater as it also demonstrated higher expression of ATP synthase muscle in low salinity water environment, but not Trypsinase. This particular family could be used to breed a line specifically raised in low salinity water.

These results provide some useful information on further research in understanding how to develop the selective breeding of shrimp for the low salinity water environments to meet the needs of aquaculture industry.

## **AREVIEW OF GLOBAL SHRIMP FARMING ISSUES AND PERSPECTIVES, AND PROSPECTS FOR INDUSTRY DEVELOPMENT IN AFRICA**

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According to FAO, global shrimp farming production reached 4.05 million metric tons (mmt) in 2011 and then increased to 4.17 mmt in 2012 (up 3%), 4.32 mmt in 2013 (up 3.6%), and 4.58 mmt in 2014 (up 6%). In contrast, the GOAL surveys indicate that world production fell to 3.87 mmt in 2012 (down 4.3%) and further down to 3.80 mmt (1.8%) for an overall decline of 6% between 2011 and 2013. GOAL data show that there was a strong rebound in 2014 (up 10% to 4.18 mmt) followed once again by a decline in production in 2015 (down 5.4%) and recovery finally taking place during 2016 - 2018 at an approximate annual rate of growth of 3.9%. Global production is expected to reach around 4.44 mmt in 2018, barring a new disease crisis.

The Asian industry appears to be recovering, after significant production declines in 2012 and 2013 caused by EMS. Production may reach 3.65 mmt by 2018, driven primarily by growth in Thailand, Indonesia, Vietnam and India. China should remain the largest producer but its contributions will be more modest. These estimates assume that no major disease will break out in the region in the next few years.

Most growth in the region will come from Ecuador, which could reach around 385,000 MT by 2018 and is increasingly targeting European and Asian markets in response to declining exports from east Asian nations. Brazil and Venezuela also expect to increase production by 2018, reaching 81,000 and 30,000 MT, respectively. Production is expected to increase from 646,000 MT in 2015 to 711,000 MT in 2018, depending on magnitude of disease impacts.

Industry trends include the need for more efficiency at every level of the production and marketing chain, industry consolidation, and creation of new markets, both internal and external. Increasing sustainable production will require more production from established and new species; expansion into new areas; improved domestication and genetic selection; new aquafeed ingredients; improved health management; new production technologies with increased control and better risk management and others. The industry also must better attract professional investors, accelerate industry consolidation, and better address the market, which will increasingly require more efficiency, quality control and traceability - through certification and sustainability of the entire production chain and sustainability.

The African continent is probably the region with the most potential for responsible growth of the shrimp farming industry (and aquaculture in general), with excellent environmental conditions and established commercial fisheries and processing plants that can support exports of farmed shrimp products. Various challenges must be addressed, including energy availability and costs, a technically-qualified labor force, investment and other issues that will be discussed.

## **PRACTICAL APPLICATION OF EXTRUSION TECHNOLOGIES FOR SHRIMP FEEDS**

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Extrusion cooking of shrimp feeds has evolved over the years and the method of production has advanced where it is quite predictable based on ingredients and product sizes. Ingredients and their combinations and the grinding characteristics play a role in the final feed characteristics. The process also requires special attention to the drying requirements as small diameter feeds have special considerations. Aspects of the extrusion production method is discussed with inputs about formulas and their relationship with the extrusion process. Methods to produce high capacity small diameter shrimp feeds is reviewed.

## DEVELOPMENT OF A FORMULATED – PELLETTED FEED FOR CRAB FARMING

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Soft-shell crab farming is a billion-dollar industry in Southeast Asia, primarily using several closely related species of the genus *Scylla*, commonly called mangrove or mud crabs. The crabs are reared in individual boxes to avoid cannibalism, with the boxes placed into a pontoon array. Multiple pontoons, controlled with a pulley system allowing the staff access to all of the boxes, float in ponds under a bridge spanning the middle of the pond which is used to stock, feed and harvest. The mangrove crabs are normally fed forage fish once a day until they molt. The freshly molted crabs are placed in fresh water so the shell will not harden and then quickly sold for local live markets or frozen for export markets. Feeding forage fish is quickly becoming uneconomic as prices, handling and storage costs are rising quickly. Feeding fish also causes fouling problems in the ponds and increases opportunities for spread of parasites and disease. Converting the crabs to pelleted feeds is needed to just maintain the present industry and critical if it is to expand.

This project first looked at various protein sources in experimental diets and then second looked at various levels of methylcellulose binder for additional pellet stability. Wild caught crabs, sized 60-80g, were collected from one estuary and delivered to a farm in Ranong, Thailand. A total of 600 crabs were randomly selected and placed into individual boxes with 150 boxed crabs stocked into each of four pontoons all in the same pond. Three experimental diets and one control diet were randomly assigned to the crabs across the four rafts. One group of 150 crabs were fed with a high fishmeal content formulated diet, another 150 with high soybean meal content diet, 150 with a new commercial crab fattening feed and 150 crabs were fed a traditional forage fish diet as the control group. Crabs were fed 3% of body weight split between two feedings a day in a 45-day feeding trial.

The commercial crab diet provided significantly better growth than the other diets, with an average growth rate of 0.89 g/day. The trash fish control was not significantly different from the high fishmeal content diet, both near 0.80 g/day. The high soy content diet was significantly lower, 0.72g/day. However, the soy based diet had the highest survival rate (95%) versus crab feed at 91% and trash fish and high fishmeal at 85% each.

The second trial with five levels of two kinds of methylcellulose (0.15%, 0.3%, 0.6%, 0.9%, 1.2%, 2.4%) was conducted in the lab with pellets in still water for one, two and three hours. Preliminary results seem to show that one type of methylcellulose was superior as a binder. The level of binder in the diet did not seem to have a significant effect within the range tested.

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## THE INDUSTRY LED R&D RESPONSE TO THE SPREAD OF PACIFIC OYSTER MORTALITY SYNDROME (POMS) IN AUSTRALIA

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The Ostreid herpesvirus type 1 microvariant (OsHV-1) is responsible for Pacific Oyster Mortality Syndrome (POMS) and first caused mortality of Pacific Oysters (*Crassostrea gigas*) in Australia in November 2010 in the eastern state of New South Wales (NSW). The virus has subsequently caused mortality in NSW every summer with infections limited to two major estuaries. In early 2016 the virus extended its reach South to Tasmania, one of the major growing regions for Pacific Oysters producing over 3,000 tonnes of oysters per year valued at AU\$23million. POMS spread rapidly through most of the major growing regions decimating over 50% of production. POMS mortalities are temperature dependent with mortalities occurring during warmer summer months. Similar levels of losses occurred again in Tasmania in the summer of 2016/17. The other major Pacific Oyster growing area of South Australia, which produces over 60% of national production, has not yet been hit by the virus but has nevertheless been severely impacted by the shortage of spat supply, the majority of which traditionally came from Tasmania. Stocking levels of spat in South Australia have reduced by over 50% with consequential shortages in supply of oysters predicted for the next two years.

Growers have responded to the virus in different ways. In NSW Pacific Oysters form only a small proportion of edible oyster production, which is dominated by the indigenous Sydney Rock Oyster (*Saccostrea glomerata*). Few Pacific Oyster producers now remain in NSW. In Tasmania growers are adapting by stocking larger numbers of spat in anticipation of higher mortalities and practicing window farming, attempting to avoid having production stock in the water, particularly the more vulnerable younger stock (<1 year), during the anticipated infection window. However, the future recovery of the industry is thought to depend on the progress of a breeding program, initiated in 2014, to breed a POMS resistant oyster. This program is predicting commercial supply of resistant oysters capable of over 70% survival in a POMS outbreak at 1 year old.

A three year research program has been initiated under the federal government's Cooperative Research Centre program, to support the industry to adapt to a future in which the virus will likely be ever present. This research program has three themes. The first of these themes promotes increased production through accelerating genetic selection for POMS resistance and production systems which reduce exposure to the virus. The second theme focuses on novel methods to assess oyster health and detect and manage oyster diseases. The third theme looks at alternative species to diversify production and innovations in Pacific Oyster production.

This presentation reports on the progress of the breeding of POMS resistant oysters and identifies the targeted research outputs that will benefit the industry in living with POMS.

## FACTORS IMPACTING PURGING SUCCESS IN DESANDING THE AUSTRALIAN PIPi (*Donax deltoides*)

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The Goolwa cockle fishery is Australia's largest such fishery, valued at over AU\$5m per annum. *Donax deltoides*, known locally as the Pipi, is a substrate dwelling bivalve which feeds on seston, primarily in intertidal zones. Traditionally the principal market for the Pipi has been as bait for recreational fishing but recently a company, Goolwa PipiCo, has been developing the seafood market for the Pipi. A key factor in accessing this market is the ability to effectively and reliably purge sand from the Pipi's after harvest. To date purging has involved transfer of the Pipi, following grading, into a recirculating aquaculture system (RAS) where it is maintained in trays for an average period of 16 hours, prior to being packaged for sale to wholesale or retail markets.

Purging success was found to be inconsistent, with some grittiness often being detected (by mouth feel) in Pipi's post-purging. There were no clear trends relating to this inconsistency of product quality. A number of factors potentially impacting on purging success were identified including location and characterisation of capture location, water quality parameters within the purging system, position of Pipi's within the purging system duration and temperature of purging systems. A methodology was developed to quantify the amount of sand in pre- and post-purged product which involves a digestion of the organic component of the Pipi.

This presentation reports on the analysis of some of the factors potentially impacting on the amount of sand in cockles, both pre- and post- purging. Data indicates that harvest site may be the most significant factor impacting on the amount of sand present in pre-purged product directly harvested from the fishery. There is also a clear relationship between the pre- and post-purging amounts of sand in the Pipis. Sand/sediment type is also being analysed and it may be that sand retention differs between different tissues of the cockle. The implications for design of efficient purging systems are discussed.

## OPPORTUNITIES, CHALLENGES AND REALITIES IN AFRICAN COMMERCIAL AQUACULTURE

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Commercial aquaculture in Africa is fraught with risk but hold significant opportunity. The co-founders of Yalelo fish farm will discuss their experience in Zambia as it relates to those opportunities, challenges and realities. The presentation will discuss issues such as estimating investment capital required, availability of capital, regulation, importance of distribution, developing technical capacity among staff and operating amid an undeveloped value-chain. Opportunities such as market dynamics, realistic production assumptions, environmental benefits and product competitiveness vs other animal proteins will also be discussed.

Yalelo Limited is Africa's largest fish farm. Since founding in 2011 and commencing production in 2013, Yalelo has installed 11,000 tonnes of production capacity and has annualized sales of 7,500 tonnes of tilapia. The fish is farmed in an off-shore floating cage installation comprised of 48 HDPE cages. The company sells fresh and frozen fish which is distributed through a network of 15 in-house stores and 30 franchises. The company supports a growing network of out-growers which supplement production.



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# APPLICATION OF THE MICROSATELLITE MARKERS IN THE SHRIMP BREEDING PROGRAMS

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Microsatellites are ideal genetic marker for applications in aquaculture. Microsatellites have properties which make them very suitable for the study of pedigree analysis in selective breeding programs. Most of the microsatellites are noncoding, and therefore variations are independent of natural selection. This paper addresses the applications of microsatellite markers in the breeding programs for closed thelycum shrimp *Fenneropenaeus indicus* in the context of management of the breeding programs. This technique was used to construct pedigree tree for the identification of the parents (Males and Females) and their offspring. A total of 100 Microsatellite markers were developed in *F.indicus*. 10 Microsatellite markers were suitable to determine the allelic inheritance and paternity within the shrimp families. All of the offspring were correctly assigned to their parents, shown to be genetic descendants of their presumed parents and showed Mendelian inheritance. The results of this paper showed that microsatellite markers are useful tool for animal breeders in genetic management of the applied breeding programs.

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# ANALYSIS OF THE GENETIC STRUCTURE OF MULLET (*Mugil cephalus*) ALONG THE MEDITERRANEAN COAST OF EGYPT USING MICROSATELLITES DNA MARKERS

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The gray mullet, *Mugil cephalus* is an important commercial marine fish species for aquaculture in Egypt. In this study, the genetic structure of the *M. cephalus* was investigated across 10 sampling sites along approximately 1,050 km of the Egypt Mediterranean coastline. Using 11 polymorphic microsatellites loci, no significant genetic structure was detected ( $F_{ST} < \text{zero}$ ), despite high genetic diversity providing sufficient power to detect structure. Overall, genetic structure for *M. cephalus* is similar to that of many marine species, which also show panmixia along this section of the Egypt Mediterranean coast. Conservation of this species in Egypt should focus on ensuring freshwater-marine migration is not diminished.

## GENETIC DIVERSITY OF MANILA CLAM (*Venerupis philippinarum*) POPULATIONS FROM LAKE TIMSAH REVEALED BY MICROSATELLITES MARKERS

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The lake Timsah is among the most worldwide productive locations of Manila clam *V. philippinarum*. Fisheries of *Venerupis philippinarum* is an important activity for livelihood of fishing villages (AL Halloos, Ezbet Adam and AL Taawin) located at Ismailia Governorates, Egypt. *Venerupis philippinarum* is an important commercially marine bivalve, and its wild populations have been severely declining in Lake Timsah during the last decade. Despite its commercial importance, genetic structure of *V. philippinarum* in Lake Timsah has not been previously investigated. In this study, microsatellites markers were analyzed on wild *V. philippinarum* populations to assess the genetic diversity and population differentiation. A total of 120 alleles were detected on 15 loci, and the number of alleles per locus in each population ranged from 2 to 8, and allelic richness per locus varied from 2.00 to 10.22 for each sample. The average of observed and expected heterozygosity ranged from 0.375 to 0.560, and from 0.553 to 0.706, respectively. Pairwise  $F_{ST}$  values indicated that all population pairs had significant genetic differentiation (overall  $F_{ST} = 0.232$ ,  $P < 0.01$ ). Cluster analysis using unweighted pair group method with the arithmetic mean (UPGMA) separated the eight populations into two groups. This study will shed light on the domestication and cultivation on population genetic diversity of *V. philippinarum*, and also provide the foundation for conservation of *V. philippinarum* germplasm resources in clam breeding practices.

## **FISH FARM WATER TREATMENT: NEW MULTI-APPROACH FOR APPLICATION OF *sodium ferrate***

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Worldwide is well known as the aquaculture sector is very promising: an important food security tool, a great business opportunity and a possibility of work for a number of technician and workers. This is not only the writer opinion but is also for other author as Peter Drucker( Management Guru) that state “Aquaculture, not the Internet, represents the most promising investment opportunity of the 21st Century.” and others.

The fast increase of the world aquaculture activities has greatly developed. Advanced technologies permit the increased densities of the farmed animals but increased the wastewater production adding some element of difficult elimination as residues, disinfectant and other molecules (antibiotics, additive, ...).

A large array of technologies is available to treat the aquaculture inlet and effluent water, to permit the appropriate, economic and to respect to the legislation framework of the aquatic farming. In fact the water used for aquaculture become a limited factor for his scarcity, cost and in competition with other human activities, the same for the land available. The above obliges a good use of the available water, appropriate water and wastewater treatment and possibly recycling with RAS (Recirculating Aquaculture System) technologies.

Ferrate (VI) is proposed as an interesting solution for a more performing aquatic animal farming. The tests already done with sodium ferrate (Fe VI) showed optimal use of this chemical for the land based aquaculture for wastewater and organic and inorganic disinfection. Until now very few authors provided information about the use of sodium ferrate in aquaculture.

Several technologies are available but the sodium ferrate shows the most promising technological characteristics, thus a new patented machinery model is presented in this paper.

The increasing concentration of farmed aquatic animals needs appropriates, clean and green technologies for water and waste water treatment to coagulate, chemical oxidation and disinfection. Ferrate (VI) is a green chemical for the above mentioned porpoises.

Now there is some patented equipment for ferrate (VI) production and utilization in coagulation, chemical oxidation and disinfection of water and wastewater treatment.

In good conditions (better acidic 6-8 PH, but still good in others), the oxidation and reduction capacity of ferrate (VI) is superior to all currently utilized oxidizers and disinfectants in water and wastewater treatment.



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## KNOWLEDGE MANAGEMENT AND INVESTING IN HUMAN CAPACITY DEVELOPMENT FOR AQUACULTURAL EDUCATION AND TRAINING IN AFRICA

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The rapid growth of the aquaculture industry, whilst driven by emerging market opportunities, has been enabled through developing knowledge and application of new scientific and technological innovations supported by private and public investments. While there are numerous initiatives directed at accessing, managing, documenting, publishing, communicating and disseminating research information and data, the present scale of 'knowledge management' is insufficient to achieve wide accessibility and use, particularly in Sub-Saharan Africa. Paradoxically, too much potentially valuable knowledge produced by committed researchers languish in libraries, unused by society; and too many of society's greatest needs for new knowledge remain relatively unexplored by researchers.

In this paper, we review recent initiatives to promote sustainable aquaculture development through improvements in education and training capacity, and innovations in the use of new web-based technologies, with emphasis on the use of digital e-learning tools. At the broad level, we present three development trends likely to shape the sector: educating for global competencies; knowledge sharing via the use of Web 2.0 technologies and open learning resources; and re-emergence of flexible and lifelong learning. The paper also presents the use of various digital e-learning platform tools and websites, that are expected to change aquaculture education and knowledge exchange.

Finally, we offer four recommendations to increase aquaculture knowledge exchange and human capacity building in Africa: (1) promote networking and mobility in aquaculture education and research; (2) develop new generic skills and competencies approaches; (3) continued professional development via e-learning and other innovative approaches; and (4) position lifelong learning in aquaculture studies.



## THE PILOT IMPLEMENTATION OF THE SEAWATER ENERGY AND AGRICULTURE SYSTEM

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Aquaculture is an increasingly diverse industry with an ever-growing number of cultured species and production systems. In the United Arab Emirates (UAE), the production from aquaculture is very small, and substantial growth is needed to reduce the existing gap between supply and demand (year 2013 estimate >136,000 tons – MoCCaE, 2015). The big challenge is that this growth occurs without creating negative environmental impacts; it has to be done in a healthy and sustainable manner. The development of environmentally sustainable aquaculture is of paramount importance for the conservation of marine ecosystems, and can be achieved by putting to good use the effluent from aquaculture activities (nutrient-rich water from organic waste), in the form of fertilizer to stimulate halophyte plant growth, in a sequential system between aquaculture, agriculture, and agroforestry.

The Seawater Energy and Agriculture System (SEAS) is an integrated food and bioenergy production process, designed to promote sustainable aquaculture, with a technology that is combined with saltwater agroforestry and agriculture. The SEAS is being implemented in a 20,000 square-meter demonstration facility, consisting of: 1) an aquaculture section, where shrimp and fish are grown to market size. The aquaculture section is made up of six open, lined ponds. Each has a capacity of about 210 cubic meters, and an overall capacity of the whole system of 1,277 cubic meters. The dedicated species for the aquaculture system were chosen according to a technical assessment, which was based on a set of preferable characteristics (favorable characters and biological features, commonly cultured organisms, etc.), and having reliable sources of juveniles. The salt tolerant *Tilapia* fish (*Oreochromis niloticus*, *O. mossambicus* and *O. andersonii*) and *Penaeid* shrimp (*Penaeus indicus*) known as Indian white prawn are the main crops for the first growing season of SEAS operation. The strategy during the second growing season of SEAS operation is to culture the most economically important species in the UAE as the main crops, such as the orange-spotted grouper (*Epinephelus coioides*) and members from the *Sparidae* family. 2) a halo-agriculture section, where the salt-tolerant and high oil-yielding plants known as salicornia are grown using the aquaculture effluent for irrigation purposes in 8 fields (685 m<sup>2</sup> each). 3) a mangrove agroforestry section that has 2 mangrove swamps (*Avicennia marina*), where excess water from the halo-agriculture section is polished and purified, while sequestering additional carbon, before recirculating the water into the system.

The SEAS facility has been operational since March, 2016. In summary, fast growth of the fish and shrimp species has been observed in the SEAS facility with very low mortality. The Salicornia and mangrove plants are showing very good growth as the nutrients are having an impact. Several research avenues are being explored.

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**SHRIMP AQUACULTURE IN EUROPE – A SUSTAINABLE FUTURE?**

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Shrimp aquaculture, in particular of *Litopenaeus vannamei* in recirculating aquaculture systems or RAS, is rapidly expanding throughout Europe. Production systems are established or being built in Bulgaria, Germany, Belgium, Italy and Switzerland to name but a few. Production in RAS can be considered sustainable in terms of nutrient outputs and in terms of water usage and wastewater outputs when compared to pond systems. RAS shrimp systems also avoid most forms of resource conflict and are extremely flexible in terms of space and time as they are not seasonally or location-limited. RAS is however energy intensive and requires significant capital inputs. Whether such systems are economically viable in the long term remains a point of intense discussion, particularly with high labour and energy costs in most European countries.

Rapid expansion is driving new research efforts. Current research in Europe is focussed on increasing potential stocking densities and improving the viability and sustainability of diet sources. In addition, research into polyculture or integrated multi-trophic aquaculture is taking place with finfish such as tilapia and even with sea cucumbers. There is also significant interest in developing replacements for eyestalk ablation, with the aim of establishing European hatcheries.

The Euroshrimp 2017 international workshop in Bremen, Germany brought together parties interested in European shrimp aquaculture. The workshop aimed to allow industry leaders to present their experience, systems and products. All participants were able to discuss current expansion plans, existing systems, lessons learned, and to identify strengths, weaknesses, opportunities and threats to the industry. An overview of some of the results of this workshop will be provided including the concerns of participants in relation to diet supply, larval supply, market (present and future) and certification. The environmental, social and economic sustainability of the industry will be reviewed.

**EFFECTS OF THE DIFFERENT DIETARY FEED ADDITIVES ON GROWTH PERFORMANCE AND NON-SPECIFIC IMMUNE RESPONSES OF JUVENILE NILE TILAPIA, *Oreochromis niloticus* AND JUVENILE OLIVE FLOUNDER, *Paralichthys olivaceus***

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An 8-week feeding trial was conducted to investigate the effects of dietary supplementation of feed additives on growth, body composition and non-specific immune responses of juvenile Nile, *Oreochromis niloticus* and olive flounder, *Paralichthys olivaceus* in fresh and sea water systems, respectively. Three experimental diets were used in each systems with 2% shrimp soluble extract (SSE), 2% squid soluble extract (SQSE) and a basal diet without feed additive as control (CONT). A number of 20 tilapias and 15 olive flounders were randomly distributed in nine rectangular 30L and 45L volume tanks, respectively. Triplicate groups of fish with initial body weight of  $4.89 \pm 0.07$  g (mean $\pm$ SD) for tilapia and  $13.4 \pm 0.13$  g (mean $\pm$ SD) for olive flounder were fed to visual satiety with one of the experimental diets twice a day. At the end of the feeding trials, weight gain (WG), superoxide dismutase (SOD) and myeloperoxidase (MPO) activity of tilapia fed the SSE diet were significantly higher ( $P < 0.05$ ) than those fed the other diets. For olive flounder, also, those fed by the SSE diet showed a significantly higher ( $P < 0.05$ ) WG, specific growth rate and SOD activity comparing to other groups. Therefore, the results of these experiments indicated that SSE in juvenile Nile tilapia and juvenile olive flounder diets have beneficial effects on growth performance and non-specific immune responses.

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**STRATEGIES FOR THE REPLACEMENT OF ARTEMIA NAUPLII IN MARINE SHRIMP DIETS**

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Hatchery production of both shrimp and marine finfish is highly dependent upon *Artemia* nauplii as a primary feed. Demand for *Artemia* cysts remains high in response to steady annual growth in the production of both shrimp and marine finfish. However, *Artemia* has several short-comings that can hinder industry advancement, including the fact that they are a limited resource. Annual production fluctuates with the weather, and annual harvests are approaching the maximum sustainable yield. The growing demand for a limited resource has resulted in escalating prices for artemia cysts, particularly when harvest from the Great Salt Lake are poor. The variable cost, supply and availability of *Artemia* cysts creates uncertainty and can be a potential bottleneck for the future growth of shrimp and marine finfish aquaculture. To continue to grow, these industries will need to find alternatives to *Artemia* in larval diets. Fortunately, shrimp larvae have evolved a requirement for the nutrients contained in *Artemia* nauplii, rather than a requirement for *Artemia* nauplii themselves. Data from commercial production trials show that an *Artemia* replacement diet that closely conforms to the nutritional composition of artemia nauplii can successfully replace 100% of the *Artemia* in larval shrimp diets with similar survival and PL quality. *Artemia* replacement diets have a number of advantages over artemia nauplii, including consistency in quality, and availability, lower product cost, lower labor cost, and reduced biosecurity risks. The present presentation presents examples of applications of a synthetic Artemia replacement diet for the hatchery production of marine shrimp postlarvae.