

# DEVELOPMENT OF LARVAE CLOWNFISH *AMPHIPRION CLARKII*: EFFECT OF SALINITY AND TEMPERATURE



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# INTRODUCTION

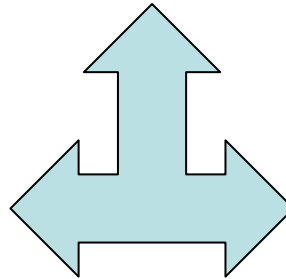
**SALINITY**



**TEMPERATURE**



Maintenance of  
aquatic organisms



Optimum ranges:  
species-specific - stage of  
development

**The interaction can enhance or lessen the isolated effect of these parameters on survival and growth**

# *Amphiprion clarkii*



- Widely distributed in tropical waters from the Indo-West Pacific
- Extremely popular in the pet trade
- Raised commercially since the early 1950s

The interaction of salinity and temperature has not been evaluated for *A. clarkii*

# OBJECTIVE

Investigate the combined effects of temperature and salinity on the larviculture of *Amphiprion clarkii*



- ✓ Find optimum condition to obtain larger and faster larval growth
- ✓ Juveniles ready for commercialization in a shorter rearing period

# MATERIALS AND METHODS



- **EXPERIMENTAL DESIGN**

**TREATMENTS:**

**20 and 30 ‰ X 26 and 30°C (6 replicates)**

- ✓ 42 larvae/2.5 L glass container
- ✓ Water bath (black bottom and sides)
- ✓ The experimental period was 17 days





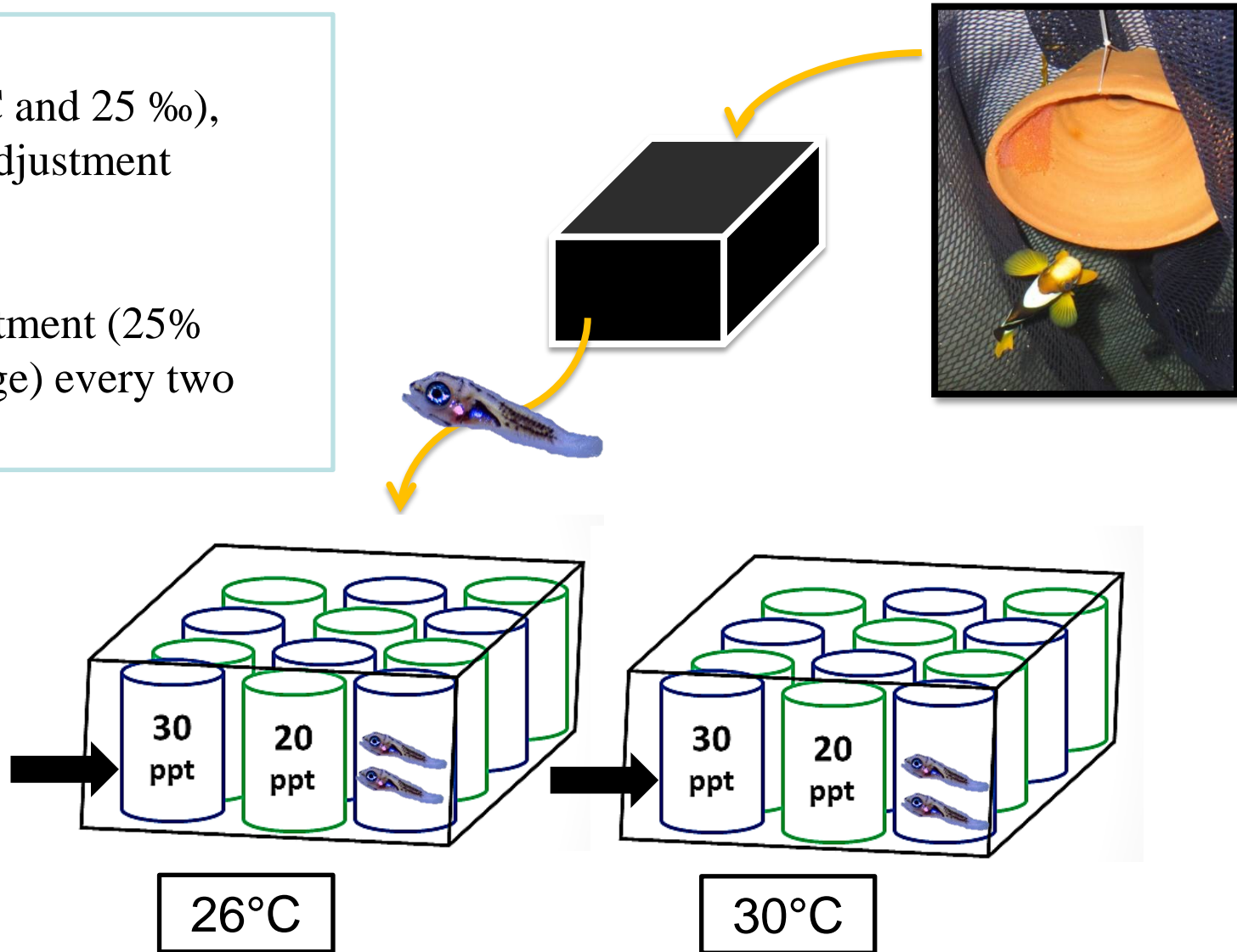
# MATERIALS AND METHODS

## 0 DAH:

- ✓ transfer (26°C and 25 ‰),
- ✓ temperature adjustment  
0.5°C.h<sup>-1</sup>

## 1 DAH:

- ✓ Salinity adjustment (25%  
water exchange) every two  
hours



# MATERIALS AND METHODS

## EXPERIMENTAL PERIOD:

Start → 1 DAH / End → 70% of metamorphosis

## Metamorphosis

- ✓ Larvae pigmentation and bands



## Maintenance conditions:

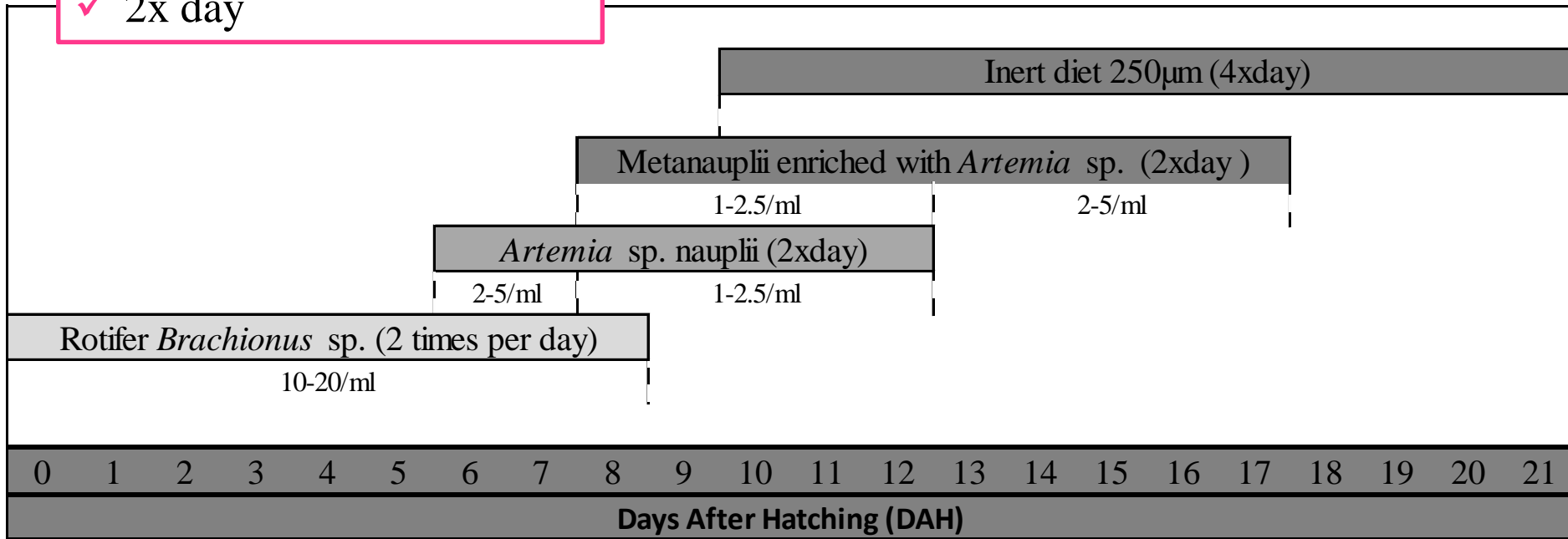
- clear water
- 30-100 % of the water volume was renewed
- 16L:8D / 2.000 LUX

# MATERIALS AND METHODS



## Feeding

- ✓ Residual count
- ✓ Residual renewal
- ✓ 2x day



- ✓ pH remained at  $8.0 \pm 0.1$  in all treatments. Nitrite ( $\text{NO}_2$ ) = 0 - 0.2  $\text{mg.L}^{-1}$ , un-ionized ammonia ( $\text{NH}_3$ ) = 0 to 0.06  $\text{mg.L}^{-1}$



# MATERIALS AND METHODS



0 DAH (16 larvae were sampled)/ end of the experiment (5 juveniles, right after undergoing metamorphosis)

## **Survival:**

✓  $S (\%) = 100 \times (N_i - N_d)/N_i$

## **Growth parameters:**

✓ Daily Growth Rate-DGR ( $\text{mm} \cdot \text{day}^{-1}$ ) =  $(T_{L_f} - T_{L_i})/t$

✓ Specific Growth Rate-SGR ( $\% \cdot \text{d}^{-1}$ ) =  $100 \times (\ln W_f - \ln W_i)/t$

✓ Weight Gain-WG (mg) =  $W_f - W_i$

## **Metamorphosis evaluation,**

- ✓ Onset of metamorphosis (OM): day in which the first juvenile appeared at each treatment;
- ✓ Pelagic larval duration (PLD): duration in days of the larval period at each treatment (metamorphosed larvae counted everyday);
- ✓ Two-way ANOVA with means compared by Tukey test with a significance level of 5 %.

# RESULTS AND DISCUSSION



## EFFECTS OF TEMPERATURE AND SALINITY INTERACTION ON PERFORMANCE PARAMETERS IN HATCHERIES *AMPHIPRION CLARKII*

Parameters	Salinity (‰)	Temperature (°C)	Interaction Salinity x Temperature
Survival rate (%)	ns	ns	ns
Total length (mm)	p<0,001	ns	ns
Wet weight (mg)	p<0,01	ns	ns
Weight gain (mg)	p<0,01	ns	ns
Specific growth rate (%/day)	ns	p<0,0001	ns
Daily growth rate (mm/day)	ns	p<0,0001	p<0,01
Start of metamorphosis (DAH)	ns	p<0,001	ns
Pelagic larval duration (day)	ns	p<0,0001	ns
Rate of larval development	ns	p<0,0001	ns

\*ns - not significant

Newly hatched larvae: total length of  $4.4 \pm 0.3$  mm / weighted  $0.7 \pm 0.0$  mg.

# RESULTS AND DISCUSSION



## Survival

- ✓ Survival of larvae was not influenced by salinity and temperature, with rates near 50 % at the end of the experiment.

The highest mortality was observed in the first week, especially on the 3rd day after hatching, decreasing gradually until metamorphosis.

Mortalities during the same period were also observed in the larviculture of the clownfish *A. akallopisos* (Dhaneesh, 2012a) and *A. percula* (Dhaneesh et al., 2009; Dhaneesh et al., 2012b). On the first two days, these authors attributed the mortality rates observed (between 10 and 20 %) to stress of larval transfer and feeding transition.

# RESULTS AND DISCUSSION

## Larval growth

### GROWTH PARAMETERS (MEAN $\pm$ SD) DURING CLOWNFISH LARVAL REARING AT DIFFERENT TEMPERATURES AND SALINITIES.

Parameters	Salinity (‰)		Temperature (°C)	
	20	30	26	30
Total length (mm)	10.1 $\pm$ 1.2a	9.3 $\pm$ 1.0b	9.6 $\pm$ 1.3	9.8 $\pm$ 1.1
Wet weight (mg)	15.9 $\pm$ 5.7a	13.0 $\pm$ 4.4b	14.2 $\pm$ 5.2	14.7 $\pm$ 5.4
Weight gain (mg)	15.2 $\pm$ 5.7a	12.3 $\pm$ 4.4b	13.5 $\pm$ 5.2	14.0 $\pm$ 5.4

Different letters at the same line for the same parameter (salinity or temperature), indicate significant differences (  $P < 0.05$  ).

Newly hatched larvae presented total length of  $4.4 \pm 0.3$  mm and weighted  $0.7 \pm 0.0$  mg.

# RESULTS AND DISCUSSION

## Larval growth

Le et al. (2009):

- ✓ Greater growth of *A. clarkii* larvae reared between 10 and 25 ‰.
- ✓ At the end of 25 days: 11.3 mm of total length at 20 ‰,
- ✓ Similar value found here for the same salinity, however at the end of 14.5 day (almost 11 days earlier).

Boeuf and Payan (2001), Baldisserotto et al. (2007): indicate that higher salinities would increase energy expenditure for osmoregulation, leading to reduced growth.



# RESULTS AND DISCUSSION

## Metamorphosis

### DEVELOPMENTAL PARAMETERS (MEAN ± SD) DURING CLOWNFISH LARVAL REARING AT DIFFERENT TEMPERATURES AND SALINITIES.

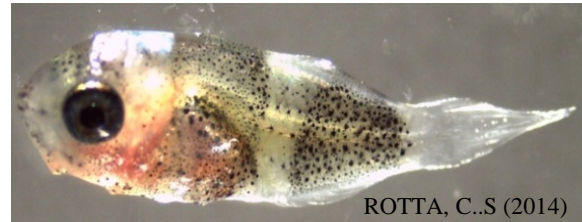
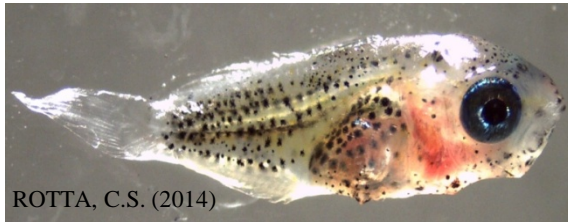
Parameters	Salinity (‰)		Temperature (°C)	
	20	30	26	30
Start of metamorphosis (DAH)	9.5±1.0	9.4±1.3	10.2±0.9a	8.8±0.9b
Pelagic larval duration (day)	14.5±1.6	13.8±1.9	15.6±0.7a	12.7±1.2b
Rate of larval development	0.07±0.01	0.07±0.01	0.06±0.003a	0.08±0.01b

✓↑T°C: 3 day difference for pelagic larval duration among the temperatures tested (12.7 days for 30°C x 15.6 days for 26°C).

# RESULTS AND DISCUSSION

## Metamorphosis

- ✓ For the ornamental mariculture → pelagic larval duration is of great importance because it is the required period to obtain juveniles ready for commercialization.



- ✓ Salinity: for ornamental mariculture, this work can help enterprises located far from the seacoast, lowering costs for seawater acquisition and reducing the occurrence of diseases and parasites.

# Conclusion

- ✓ Survival was not affected by different salinities and temperatures, and is within the expected range for this species as observed in other studies. However, growth was influenced by both factors.
- ✓ Higher growth as length, weight and weight gain: 20%. Optimization of larval development: 30°C (earlier onset of metamorphosis -8,8 DAH, and lower larval duration-12.7 days) compared to 26 °C.
- ✓ Therefore, under the conditions used, the larval rearing of clownfish *Amphiprion clarkii* can be maximized at higher temperatures (30°C) and lower salinity (20 ‰).



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Obrigada!