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# Effect of salinity on the embryonic development of *Macrobrachium acanthurus* (Decapoda: Palaemonidae)

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**ABSTRACT**

The effect of salinity level on the embryonic development of *Macrobrachium acanthurus* was analyzed under laboratory conditions, considering characteristics of the egg (size, volume, and water content) and of the embryo (eye index). The experimental design was completely randomized, with five repetitions (ovigerous females) per treatment (0, 10, 17 and 20 ppt). During embryonic development, two eggs per female were taken daily for analyses of size, volume, water content, and eye index. Our results showed that salinity of 20 ppt leads to death and/or abortion of the embryo in all females. The size, volume and water content of eggs increased according to embryonic development, providing space in the egg for formation and organization of embryo. Salinity affected these egg characteristics, causing water loss to the hypertonic medium. Neither the duration of embryonic development nor embryo formation were affected by saltwater content. The results of the present study indicate that ovigerous females of *M. acanthurus* can survive in freshwater rivers as well as in low-salinity environments during incubation period and the successful larval development is not likely to rely on female migrating to estuaries. Larvae can easily be incubated in freshwater and complete development at higher salinities after hatch.

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Freshwater prawn; embryology; egg volume; water content; eye index

## Introduction

Most caridean shrimp live in the marine environment, with only 25% of 3200 species inhabiting freshwater (De Grave et al. 2009). Although several species inhabit brackish water, they are not considered exclusively estuarine due to the transient condition of areas such as mangrove regions, estuaries and river mouths. These freshwater carideans complete their larval development in brackish water, and juvenile adults exhibit vagile features and the capacity for osmoregulation, which allows organisms to move freely between freshwater and estuarine environments (Bueno & Rodrigues 1995).

Freshwater prawns can be separated into two groups according to the environment in which they spend most of their life cycle. The first group comprises amphidromous species, in which females incubate numerous small eggs; organisms exhibit extended larval development and depend on brackish water; and, after metamorphosis, juveniles migrate upstream to integrate with local populations. In the second group, females incubate fewer large eggs; lecithotrophic larvae hatch and present benthonic habits; and larval development generally involve only two or three zoea instars (Magalhães & Walker 1988; Jalihal et al. 1993; Bauer 2011).

Salinity is an important environmental factor that imposes selective pressure on aquatic organisms, and the ability of crustaceans to survive in different salinities depends on several adaptations (Anger 1995). The salinity level and the nature of its variation impact the composition and osmolality of animal body fluids, and some species exhibit a physiological capacity for osmoregulation (Charmantier & Charmantier-Daures 2001). Osmoregulation is a process by which organisms maintain a concentration of solutes that is stable in relation to the environment through the active transport of ions, and it is one of the most effective mechanisms for adaptation among aquatic species (Berger & Kharazova 1997; Vega-Villasante & Carrillo 2006).

The physiological mechanisms that allow decapods to tolerate fluctuations in salinity levels have been studied in both adults and larvae (Anger 2001, 2003), but few studies have investigated the effects of salinity on embryonic development (Charmantier 1998; Susanto & Charmantier 2001; Ituarte et al. 2005).

Tolerance of the stress response to changes in salinity varies during ontogeny (Kinne 1971). Juvenile and adult crustaceans are generally more tolerant to salinity changes than are embryos and larvae (Greenwood et al.

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1989); salinity levels can affect embryonic development, oogenesis and larval quality (Crisp & Costlow 1963; Bas & Spivak 2000; Gimenez & Anger 2001).

Information on salinity tolerance at various stages of decapod embryonic development is scarce, but several studies have been performed on crabs: *Carcinus maenas* (Linnaeus, 1758) (Hartnoll & Paul 1982; Anger et al. 1998), *Chasmagnathus granulata* Dana, 1851 and *Cyrtograpsus angulatus* Dana, 1851 (Bas & Spivak 2000; Gimenez & Anger 2001).

Most studies on palaemonids have focused on the effect of salinity in relation to biological and physiological aspects. The studies published have investigated the embryonic development of *Palaemonetes argentinus* (Nobili, 1901) (Ituarte et al. 2005); the osmoregulatory ability and metabolism of *Macrobrachium acanthurus* (Wiegmann, 1836) (Moreira et al. 1982, 1983; Araujo & Castro 1985), *M. heterochirus* (Wiegmann, 1836) (Moreira et al. 1982, 1983); *M. olfersii* (Wiegmann, 1836) (McNamara et al. 1982; Moreira et al. 1982, 1983; McNamara 1987) and *M. potiuna* (Muller, 1880) (Moreira et al. 1983); the respiratory metabolism, survival and larval molt of *M. amazonicum* (Heller, 1862) (McNamara et al. 1983); the oxygen consumption rate of *M. acanthurus* (Gasca-Leyva et al. 1991); the osmoregulation and oxygen consumption of *M. tuxtlaense* Villalobos & Alvarez, 1999 (Ordiano et al. 2005); the physiological adaptation (Agard 1999) and fertility of *M. rosenbergii* (De Man, 1879) (Yen & Bart 2008); the growth and survival of *M. tenellum* (Smith, 1871) juveniles (Vega-Villasante et al. 2011), and the effects of salinity and temperature on the survival and development of *M. lar* (Fabricius, 1798) (Lal et al. 2012).

*Macrobrachium acanthurus* is a freshwater shrimp that is widely distributed, from the eastern basins of the United States to southern Brazil (Melo 2003). The species is of commercial interest with great potential for cultivation (Valenti 1985). Although *M. acanthurus* spend most of their life cycle in freshwater, the species exhibits extended larval development, which depends on brackish water for completion. Under laboratory conditions, larval development occurs successfully at salinities of 15–20 ppt (Choudhury 1971). Planktonic larvae pass through 9–11 larval stages in estuaries, taking 30–40 days to complete metamorphosis and then migrate to freshwater (Choudhury 1970; Valenti 1985; Bertini & Valenti 2010; Bertini et al. 2014).

As *M. acanthurus* is present in almost all rivers of the Brazilian coastal region that drain to the sea, the pattern of its development may change according to geographic location and the habitat of ovigerous females, i.e. a completely freshwater environment or nearby seawater with varying salinity levels. The objective of this study was to analyze the effect of salinity level during embryonic development in *M. acanthurus* under laboratory conditions, considering the characteristics of the embryo (eye index) and egg (size, volume and water content). We aimed to test whether increased salinity affects the embryonic development of *M. acanthurus,* leading to a delay or a change in ontogeny.

## Methods

The experiments were conducted at the Laboratory of Biology and Cultivation of Crustaceans (LABCRUST) in the São Paulo State University 'Júlio de Mesquita Filho' (UNESP) – Registro to properly monitor the embryonic development of *M. acanthurus.* For the development of each experiment about 60 shrimp were collected using traps and sieves at Ribeira de Iguape River, which is located in the city of Registro, in the Vale do Ribeira region, south of state São Paulo. The size of individuals ranges from 15 to 20 mm of carapace length. The animals were transported in buckets containing freshwater from the collection site to the LABCRUST.

In the laboratory, shrimp were separated according to sex based on morphological characteristics, with males recognized by the presence of a male appendage in the second pair of pleopods and females by the absence of this appendix according to Bertini et al. (2014). Ovigerous females were used to investigate embryonic development in the laboratory. For this, shrimp with developed gonads (considered able to reproduce) were placed in glass aquariums (16 × 24 × 17 cm) with a sex ratio of one male for two females. Tanks were filled with freshwater and maintained at 29 °C. The substrate was composed of coarse sand, rocks, and vegetation to simulate a natural environment. Black plastic covered the sides of the aquaria to prevent movement in the laboratory from interfering with the copulatory behavior of the shrimp.

Individuals were fed daily with fish and diet prepared in the laboratory according to Barros and Valenti (2003), in order to fulfill all shrimp nutritional demands and provide adequate gonadal development. Tank water was exchanged frequently, and food debris was removed to maintain water quality. The photoperiod was controlled with 14 h light and 10 h dark. The shrimps were monitored daily to detect ovigerous females. When detected, ovigerous females were transferred to the aquariums in which the experiments were to be conducted. Females were kept isolated until the time of larvae hatching in all experiments, which allowed researchers to monitor the eggs throughout the embryonic period.

### *Experiment 1. Influence of salinity on egg size, egg volume, and eye index*

For this experiment, 20 ovigerous females were gradually acclimatized in freshwater (0 ppt) by increasing the salinity

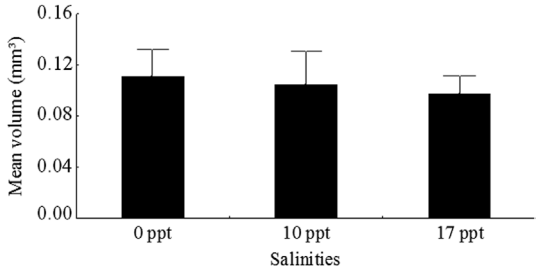


**Figure 1.** Mean egg volume (±SD) during the embryonic development of *Macrobrachium acanthurus* at various levels of salinity.

level 5 ppt every 2 h until the desired level was reached. The experimental design was completely randomized, with four treatments (0, 10, 17, and 20 ppt) and five replicates (ovigerous females) per treatment, with a total of 20 replicates.

For the size analysis, two eggs were removed from each female every day, during the entire incubation period. These eggs were measured with respect to larger diameter (LD = funiculus at the opposite margin) and smaller diameter (SD = between lateral margins, opposite to the funiculus). The LD/SD ratio was calculated for each measured egg by determining the mean values and standard deviation. The confidence interval was calculated to determine egg shape ($\alpha = 0.05$): spherical (LD/SD = 1) or ellipsoid (LD/SD ≠ 1). Differences in egg size were tested with a 95% confidence interval. Egg volume was calculated using $V = 4/3\pi r^2 R$ (where $r$ = smallest radius = SD/2; $R$ = larger radius = LD/2) (Odinetz-Collart & Rabelo 1996).

Eye index ($I$) was calculated by measuring the larger ($L$) and lower ($l$) lengths of the embryo eye, using $I = (L + l)/2$. This methodology was utilized after the initiation of eye pigmentation (Perkins 1972).

### *Experiment 2. Analysis of egg water content at various salinity levels*

For this experiment, another 20 ovigerous females were gradually acclimatized in freshwater (0 ppt) to the salinity levels tested (10, 17 and 20 ppt). During the entire incubation period, spawning to hatching, a small amount of eggs was removed from each female to determine the water content of the egg mass. For fresh-weight analysis, the egg sample was removed from females, placed in aluminum foil, weighed on precision scales (0,001 g) and subsequently taken to a 60 °C oven for 48 h to determine dry weight, as described by Clarke (1993).

The water content of the egg mass was calculated using (FW – DW) × 100/FW, where $A$ corresponds to the water content of the sample, FW corresponds to the fresh weight and DW to the dry weight (Clarke 1993).

### *Statistical analysis*

The duration of embryonic development was tested at different salinities of 0, 10, 17 and 20 ppt. Egg volumes, eye indexes and water contents were tested for four salinity levels and to the day of embryonic development. All analyses were performed using SAS software for Windows (version 9.2). Data were tested for homoscedasticity and normality, and the General Linear Model (GLM) with Tukey's test for multiple comparisons of means were applied to all analyses.

## Results

The data for the 20-ppt salinity treatment from both experiments were not used in the analysis, as none of the ovigerous females completed embryonic development. The females died or aborted the embryos; sometimes abortion of the embryo was followed by death of the female, indicating that this species does not survive at a salinity level of 20 ppt.

In the other treatments, the timing of embryonic development showed no statistically significant difference among salinity levels ($p > 0.05$), with a mean time of 12.5 (±0.8) days. Final egg size varied according to environmental salinity. In freshwater, egg size reached 0.79 mm (greater length of the ellipse) on the last day of embryonic development. At higher salinity levels (17 ppt), egg size reached 0.77 mm. *Macrobrachium acanthurus* eggs exhibited an ellipsoid shape throughout embryonic development, as evidenced by a DMA/DME ratio ≠ 1. Egg volume differed statistically at the salinities tested ($p < 0.05$) (Figure 1), i.e. egg volume decreased with increasing salinity (Table 1). However, regardless of salinity, this volume increased during embryonic development, by 41.7% (0 ppt), 49.4% (10 ppt), and 35.9% (17 ppt), respectively, from the beginning to the end of development.

The growth pattern of egg volume as observed in freshwater, which represents the species' natural environment, can be defined as $V = 0.072 + 0.0057 \times T$, where $V$ = volume ($mm^3$) and $T$ = time. At salinity levels of 10 ppt and 17 ppt, egg volume can be estimated by $V = 0.05719 + 0.007115 \times T$ and $V = 0.07299 + 0.0036 \times T$, respectively, with a high determination coefficient ($r^2 = 0.93$).

Egg water content exhibited statistically significant differences between salinity levels investigated ($p < 0.05$). From the beginning to the end of embryonic development, egg water content showed a 26.5% increase in fresh water, a 12.9% increase at 10 ppt salinity and a 7.04% increase at 17 ppt salinity. However, after analysis of egg water content for each salinity level, there was no statistical difference in the egg water content during embryonic development ($p > 0.05$) which demonstrates that water

**Table 1.** Features of the egg and embryonic development in freshwater prawns of the genus *Macrobrachium*.

| Species | Salinity (ppt) | Development larval | Temperature (ºC) | Mean duration of embryonic development (days) | Egg size (mm) | Egg volume ($mm^3$) | Egg water content (%) | Eye pigmentation/ eye index | References |
|---|---|---|---|---|---|---|---|---|---|
| *M. acanthurus* | 0 | Extended | 29° | 12.5 | 0.58–0.79 (L) onset-end | 0.11 (±0.02) | 71.22 | 60–160 µm | Present study |
| | 10 | Extended | 29° | 12.5 | 0.58–0.85 (L) onset-end | 0.10 (±0.02) | 64.7 | 60–160 µm | Present study |
| | 17 | Extended | 29° | 12.5 | 0.58–0.77 (L) onset-end | 0.09 (±0.01) | 60.3 | 60–160 µm | Present study |
| *M. acanthurus* | 0 | Extended | 25° | 16 | | 0.075–0.109 | – | 150.3 µm (end) | Müller et al. (2007) |
| *M. acanthurus* | 0 | Extended | – | – | 0.73 (L) × 0.57 (l) | – | – | – | Anger & Moreira (1998) |
| *M. amazonicum* | 0 | Extended | – | – | – | 0.14–0.27 | – | – | Odinetz-Collart & Rabelo (1996) |
| *M. olfersi* | 0 | Extended | 25° | 14 | – | 0.0229–0.0720 | – | 50–64% of embryonic development | Müller et al. (2003) |
| *M. olfersi* | 0 | Extended | – | – | – | 0.076 | – | – | Corey & Reid (1991) |
| *M. ohione* | 0 | Extended | – | – | – | 0.08 | – | – | Corey & Reid (1991) |
| *M. ohione* | 0 | Extended | 22–23 | 18 | – | – | – | – | Bauer & Delahoussaye (2008) |
| *M. carcinus* | 0 | Extended | – | – | – | Stage 1 – 0.066 Stage 3 – 0.088 | 66.3–76.8 | – | Lara & Wehrtmann (2009) |
| *M. rosenbergi* | 0 | Extended | 28.5 | 20 | 0.68–0.79 (L) | – | | 8th day of incubation | Habashy et al. (2012) |
| *M. lamarrei* | 0 | Extended | 30 | 16 | Mature egg (end) 0.54–0.64 | – | – | – | Sharma & Subba (2005) |
| *M. borelli* | 0 | Abbreviated | 24 | 39 | 1.5–2.0 | – | 51.2–61.5 (based on dry weight) | Stage 2 (8–15 days): 1.02–1.13 (L) | Lavarias et al. (2002) |
| *M. lanchesteri* | 0 | – | – | – | Without eye: 0.8 × 0.7 With eye: 1.0 × 0.8 | – | – | – | Phone et al. (2005) |
| *M. hainanense* | 0 | Abbreviated | 20–22 | 53.4 | Without eye: 2.03 × 1.47 With eye: 2.11 × 1.55 | Without eye: 2.31 With eye: 2.68 | – | – | Mantel & Dudgeon (2005) |
| *M. americanum* | 0 | Extended | 24 | 18 | – | – | – | 30–40% (7–8 days) of embryonic development | García-Guerrero & Hendrickx (2009) |
| *M. idella idella* | 4–5 | Extended | 27–28 | 13–14 | 0.47–0.65 | – | – | 156 h | Dinakaran et al. (2013) |

Note: Considering: Larger length (L) and smaller length (l).

content does not influence the embryonic incubation period (Figure 2).

Eye development in the embryos was initiated on the 7th day after fertilization. The eye index increased with embryonic development and showed a statistically significant difference ($p < 0.05$) over the days. The eye index did not differ statistically among salinity levels (Figure 3), which suggests that embryo formation does not vary with environmental salinity. The eye index for *M. acanthurus* varied from 60 to 160 micrometers between the beginning and the end of development. This index can be estimated by $I = 0.049 + 0.016 \times T$ ($r^2 = 0.84$), where $I$ = eye index and $T$ = time.

## Discussion

Based on the experiments conducted in this study, salinity levels of approximately 20 ppt can limit the establishment

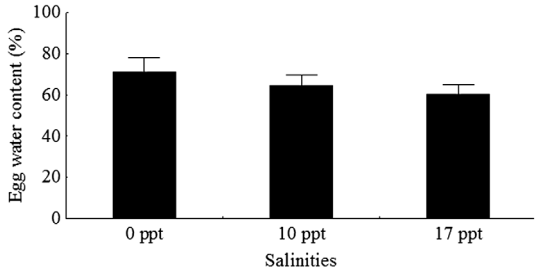


**Figure 2.** Mean values of egg water content (±SD) during the embryonic development of *Macrobrachium acanthurus* at various salinity levels.

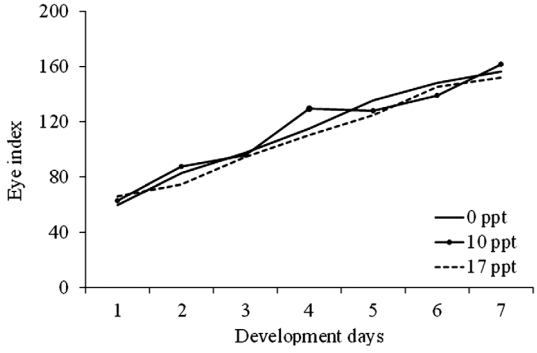


**Figure 3.** Mean values of the eye index during the embryonic development of *Macrobrachium acanthurus* at various salinity levels.

of an adult population of *M. acanthurus,* despite the species' ability to inhabit estuaries. All animals in the experiments performed at this salinity level died, which may be related to the duration of time for which shrimp can tolerate that level of salinity. Similar results were obtained by Signoret and Brailovsky (2004) in Mexico for *M. acanthurus*, where this species showed hyperosmotic regulation in salinities between 0 and 20 ppt, while at higher salinity levels (21–35 ppt) animals could not perform osmoregulation and exhibited high mortality rate. Notably, the larvae of this species require salinity values of approximately 15 ppt for larval development to occur properly (Choudhury 1970).

For organisms that prefer freshwater, high salinity values require high energy investment to maintain osmoregulation, which is disadvantageous for other physiological processes (Vijayan & Diwan 1995). When *M. acanthurus* is exposed to 21 ppt salinity, important physiological processes are altered; there is an increase in the oxygen consumption rate, which can reach 1.11 µl $O_2$ mg dry weight$^{-1}$.h$^{-1}$ (Moreira et al. 1983). Although *M. acanthurus* presents a high isosmotic point (between 632 and 640 mOsm kg$^{-1}$), which confers a greater tolerance to salinity changes (Moreira et al. 1983; Signoret & Brailovsky 2004), the stress induced by high salinity exposure (20 ppt) may have contributed to embryo abortion and death of the females.

The duration of embryo development for *M. acanthurus* in the present study was approximately 12 days at 29 °C. However, in southern Brazil, this species exhibited extended development: 16 days at 25 °C (Müller et al. 2007). This difference may be related to the water temperature during the incubation period, because higher temperatures increase the speed of metabolic processes and thus embryo development.

The incubation period is also related to the type of larval development observed. *Macrobrachium acanthurus* has a short incubation period, similar to that of *M. olfersii* (Müller et al. 2003), *M. lamarrei* (H. Milne Edwards) (Sharma & Subba 2005), *M. americanum* Bate, 1868 (García-Guerrero & Hendrickx 2009), *M. idella idella* (Hilgendorf, 1898) (Dinakaran et al. 2013), and *M. lar* (Lal et al. 2014). Species such as these with extended larval development tend to exhibit many larval stages and depend on the estuary with moderate salinities or from marine ambient to complete development (Bauer 2004; Lara & Wehrtmann 2009; Lal et al. 2012, 2014). However, some *Macrobrachium* species present abbreviated larval development, and females incubate embryos for a longer period, as in *M. hainanense* (Parisi, 1919), which has an incubation period of up to 53 days (Mantel & Dudgeon 2005).

There was an increase in egg size at all tested salinities throughout the embryonic development of *M. acanthurus*. This growth during development is a pattern for crustaceans and is associated with changes in egg shape (Pinheiro & Hattori 2002). Egg diameter tends to increase until hatching, as gradual yolk addition by embryonic cells provides space in the egg for the development of body appendages, abdominal growth and organization of the organs in the cephalothorax (Odinetz-Collart & Rabelo 1996; Müller et al. 2003).

Water content levels remained constant during the ontogeny of *M. acanthurus,* in contrast to the ontogeny of *M. carcinus* (Linnaeus, 1758) (Lara & Wehrtmann 2009), which involves an increase in egg water content ranging from 66.3 to 76.8% until the end of the incubation period. With increasing salinity, there was a reduction in egg volume and water content, because the environment becomes hypertonic in relation to the egg mass.

The increase in egg volume is an important factor in embryogenesis at the end of the incubation period, allowing greater mobility of the embryo as well as larval hatching (Kobayashi & Matsuura 1995; Müller et al. 1999; Nazari et al. 2000). This increase may be associated with the natural growth of the embryo (Müller et al. 2003), with the growth of the embryonic structures in the cephalic–caudal axis (Anderson 1982), as well as the retention that results from respiration metabolites and lipid reduction

in the eggs (fatty acids), which in turn provides energy for embryonic development (Clarke et al. 1990).

The eye index increased until the end of embryonic development at all tested salinities, demonstrating that embryo growth is constant throughout egg incubation. This increase in the eye index of *M. acanthurus* corroborates findings reported for the crab *Erimacrus isenbeckii* (Brandt, 1848) (Nagao et al. 1999) and for the lobster *Homarus americanus* Milne Edwards, 1837, which showed an increase by 490 µm during incubation (Perkins 1972). An increase in the pigmentation percentage as development proceeds was already observed in palaemonids as *M. olfersii* (Müller et al. 2003; Simões-Costa et al. 2005), *M. potiuna* (Müller et al. 2004), *M. idella idella* (Dinakaran et al. 2013) and *M. lamarrei* (Rashid et al. 2013) (Table 1).

In the present study, the eye index was constant at different salinities (10 and 17 ppt), which suggests that the embryonic development of this species is not affected by salinity at the levels tested. Therefore, during the incubation period, the ovigerous females of *M. acanthurus* can remain in freshwater rivers as well as in environments with some marine influence, with no need for displacement into the estuary for the purpose of hatching larvae. This result supports findings published by Bertini et al. (2014), which showed that there is no large-scale migration of adult *M. acanthurus* to estuaries during the reproductive period.

The present study showed there is no change in the embryonic development of the freshwater prawn *M. acanthurus* at salinities of 10 and 17 ppt. This allows for the species' wide distribution: it inhabits almost all Brazilian coastal rivers that drain to the sea. However, regions with salinity of 20 ppt may be considered limitrophic for species establishment. The information generated by this study has great value in the context of research concerning cultivation technology for this species of important economic interest, because it would allow broodstock maintenance in freshwater, with no need to acclimate ovigerous female in brackish water during incubation period. Therefore, it would make easier to manage creation of this species which has great aquaculture potential.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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