

# The Effect of Salinity on the Embryonic Development of the Giant Freshwater Prawn *Macrobrachium rosenbergii* (De Man, 1879) Under Laboratory Conditions

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**Abstract:** Prawn farming is a highly profitable activity given the high level of commercial interest associated with prawn species. *Macrobrachium rosenbergii*, known as the giant freshwater prawn, is the largest prawn of its genus and presents great adaptability in terms of the handling it requires. Given the foregoing, research is ongoing to broaden knowledge in order to improve the quality of production for this species in northern region of the state of Veracruz, Mexico, by studying the behavior of these anadromous organisms from the first stages of life onwards. The experimental design of the present study involved placing gravid females in incubators with different levels of salinity (5, 9, and 12 psu) and a control containing freshwater, with three replicas conducted per salinity treatment, giving a total of 12 experimental units with a female in each. In general terms, the results obtained showed, in general terms, a total of 13 days for the embryonic development of *M. rosenbergii*. Although a decreasing volume of eggs was observed as the salinity increased, the percentage of hatchings was higher at 9 psu, with 65%, than for the other salinity treatments. The results also reveal that the 12 psu treatment obtained a better survival percentage (12.06%) than the other treatments. The different biotic factors to which the organisms may be exposed are also considered in the present study.

**Keywords:** *Macrobrachium rosenbergii*, Embryonic Development, Larvae, Salinity

## 1. Introduction

Over recent years, aquaculture has grown significantly due to its increasing importance as an alternative to fishing, as it has a lower environmental impact. In light of estimates that, by the year 2050, demand for aquaculture resources will stand at approximately 80 million tons [3], interest in improving the quality of aquatic organism cultures has increased, leading to the search for more knowledge on the processes involved in order to increase production. It should be noted that crustaceans now represent a high percentage of the commercial value of aquaculture in Mexico [27]. The genus of the *Palaemonidae* family that attracts the most interest is *Macrobrachium*, due to the commercial value of its biological characteristics, such as its size and weight, and

environmental characteristics, in that the genus occurs in the tropical and subtropical regions of the world [32]. According to [17, 33], ten *Macrobrachium* species occur in Mexico, of which solely five are of commercial value: *Macrobrachium americanum* (Bate, 1868) and *Macrobrachium tenellum* (Smith, 1871), occurring in the Pacific Ocean and the Gulf of Mexico; *Macrobrachium acanthurus* (Wiegmann, 1836); *Macrobrachium carcinus* (Linnaeus, 1758); and, as an exotic species, *Macrobrachium rosenbergii* (De man, 1879), which is found in aquaculture farms in different regions of the Mexican Republic. Commonly known as the giant freshwater prawn or the giant river shrimp, *Macrobrachium rosenbergii* is subject to great commercial demand [29].

Growing to a maximum total length of 320 mm for males and 250 mm for females [17], this species, the largest of its

genus and native to the Indo-Pacific, was introduced to Mexico due to its size and easy adaptability to farming [23]. After reproducing in freshwater and requiring salinity for their life processes [25], prawn species descend to brackish regions to hatch their eggs and continue their larval development [24]. With osmoregulation playing a fundamental role in embryogenesis, various studies have found that the embryonic membrane of crustaceans is responsible for protecting osmosis during embryogenesis, in relation to/via/as a function of increased levels of the enzyme Na<sup>+</sup>/K<sup>+</sup>-ATPase [5]. Therefore, research has sought to identify the optimal salinity requirements, observing the growth of *M. rosenbergii* embryos at different salinity levels, in order to ascertain how this physicochemical parameter influences the development of the embryo and to identify the ideal salinity level for a healthy organism with the desired characteristics. This information would enable producers to improve their management of cultures of this species and, thus, obtain organisms of a higher quality.

Reported the first observations of the importance of saline water to the early life stages of *M. rosenbergii*. [16] In a study comprising six salinity treatments (0, 6, 9, 12, 16, and 18 psu) with three replicas each and an average embryonic duration of 22 days, [4], found that embryonic development tended to decrease as salinity increased. They also found an optimal salinity range of 12 psu, given that this range presented the highest survival percentage. [28], report that *M. malcolmsonii* embryos incubated *in vitro* hatch better at a minimum salinity of 0.5 psu than in salinities of 2, 4, 6, 8, and 10 psu, observing mortality in the first three embryonic stages and concluding that 0.5 psu is a good level of salinity.

Carried out a study in Argentina on the effect of salinity on the embryonic development of two species of estuarine crabs (*Chasmagnathus granulata* and *Cyrtograpsus angulatus*) of the South-West Atlantic, which are cultivated *in vitro* due to the fluctuations in salinity present along the Mar del Plata coast. Working with salinities ranging from 12 to 44 psu, they observed that the egg tends to shrink as salinity increases, thus identifying that the volume of the embryos was inversely proportional to the salinity to which they were exposed. [2]

Developed a manual on *M. rosenbergii* for the Food and Agriculture Organization of the United Nations (FAO), which indicates that maintaining a salinity of 5 psu and a pH of 7.0 to 7.2 for gravid females until hatching ensures an improved hatching rate [25]

Carried out two treatments for prawns from the Gulf of Mexico, *M. acanthurus* and *M. carcinus*. After applying both drastic and gradual changes in salinity, they observed significant differences in mortality and a strong relationship between the survival of these species and the amount of saline water supplied to the medium [30]

In research conducted in Chile, [34], studied the daily embryonic development of the river prawn *Cryphiops caementarius* (Decapoda: *Palaemonidae*) under laboratory conditions. Applying three different temperature treatments, they conducted daily embryo (3–6) extraction on each female

for subsequent observation under the microscope, identifying a total of seven embryonic stages for this species. [10], assessed, *in vitro*, the effect of salinity on early developing *C. caementarius* embryos taken from one single female, applying four treatments (0, 10, 20, and 30 psu) in 400 ml incubators, with three corresponding replicas comprising 200 embryos each. Salinities of 0 and 30 psu did not affect the development of the embryos until the sixth embryonic stage, while a delay in the presentation of stages seven and eight was observed for the 10 and 20 psu treatments. They found that a salinity of 30 psu caused death at Stage 7, while, at 0 psu, 70.3% of the embryos survived and the larvae were normal; however, at 10 psu and 20 psu, 13.5% and 7.9%, respectively, of the larvae were able to hatch, although they did present deformations. [14], in research conducted in Egypt, described the embryonic changes occurring during the development of *M. rosenbergii*, reporting an embryonic duration of 20 days, although it should be taken into account that, in their study, the eggs were placed in freshwater.

In Peru [1] conducted a study on the embryonic development of *M. americanum*, placing gravid females in incubators and gradually increasing the salinity by 1 psu increments until reaching 13 psu. They found that embryogenesis lasted for 17 days at 28 °C, after which, at the point of hatching, the larvae were placed in 5 L containers with salinity levels of 1, 12, 16, 20, 24, and 26 psu. They observed mortality at 1 psu and 100% survival levels at the other salinity levels. This finding highlights the importance of salinity for larval metamorphosis, wherein the higher the salinity, the higher the survival rate. However, the feed (*Artemia* sp. nauplii) used in the foregoing study was inadequate, with the individuals studied solely reaching the third zoeal stage.

## 2. Material and Methods

The present study was conducted in the aquaculture laboratory of the Postgraduate College, Campus Veracruz, in the state of Veracruz, Mexico. Fifty breeding individuals were selected at a ratio of 1:3 (male / female) and placed in freshwater in a 4,580 L circular geomembrane tank, 2.70 m in diameter and 80 cm high, with oxygenation supplied by means of a ½ HP blower motor, with 15-20 cm long PVC tubes, two inches in diameter, then introduced as a shelter for the individuals. Green burlap was also used to subdivide the tank along its length to provide a mating area for the males. The specimens were fed once a day with pelletized commercial shrimp feed (of the brand *El Pedregal*), at a 40% ratio, and, eventually, tilapia fillet, viscera, and fresh gills.

Once the females had been fertilized, a biometry was performed to obtain their corresponding length and weight, measuring from the tip of the rostrum to the rear end of the telson for the total length [14], thus enabling the calculation of the weight and length ratio for females both with and without eggs.

Three treatments were applied, with salinities of 5, 9, and 12 psu, while a fourth freshwater (0 psu) treatment served as

the control. [15] Three replicas corresponding to each of the three treatments were carried out, giving a total of 12 experimental units with one female per replica.

The ovigerous females were placed in 65 L rectangular tanks [18], each of which was equipped with an oyster shell-based cascade-type biological filtration system, a fine mesh bag used as netting to avoid leaks at the base, a thermostat, and an aquarium aerator stone. The saline water was transported directly from Mata de Uva Beach, in the municipality of Alvarado, in the state of Veracruz, and was disinfected with 0.5 ml sodium hypochlorite for each liter of saline water and then aerated for 24 h. Five drops of sodium thiosulfate pentahydrate was then added per liter of water to remove the residual chlorine. The embryos carried by each ovigerous female were then checked every 72 hrs to prevent possible stress from affecting egg development.

The pH level was measured every morning, registering values of  $7 \pm 0.4$ , while the dissolved oxygen level was recorded at  $4.15 \pm 1.40$  ppm and the temperature was  $28.2 \pm 3.0^\circ\text{C}$ .

The morphological description of the embryos was undertaken based on the following studies on the embryonic development of palaemonids: [16, 20, 21, 10, 12, 14, 9]; and, [19]. The development phase was expressed as a percentage, according to the criteria described by [11]. Using the Image j digital processing program, the width (a) and length (b) of the eggs was obtained and then entered into a database produced using Excel software, while the volume (v) of ellipsoid bodies was calculated using the following formula:

$$v = \frac{\pi}{6} \cdot b^2 \cdot a \text{ [31].}$$

**Table 1.** Biometry of *M. rosenbergii* breeding females by treatment.

Treatment	Replicas	Length of female with eggs (cm)	Weight of female with eggs (gr)	Length of female without eggs (cm)	Weight of female without eggs (gr)
1 (0 ups)	1	12	19	12	17
1 (0 ups)	2	13.5	23	13.5	20
1 (0 ups)	3	12.7	23	12.7	22
1 (5 ups)	1	11.5	22	11.5	20
1 (5 ups)	2	11.5	24	11.5	23
1 (5 ups)	3	11.3	24	11.3	23
1 (9 ups)	1	13.8	32	13.8	30
1 (9 ups)	2	14	38	14	34
1 (9 ups)	3	12.5	24	12.5	22
1 (12ups)	1	12.5	26	12.5	24
1 (12ups)	2	10.5	18	10.5	17
1 (12 ups)	3	12.3	24	12.3	22
		12.3±1	24.8±5.4	12.3±1	22.8±4.8

The egg mass presented different tones as the embryo developed, with a bright orange coloration observed on Day 1, which, by Day 4, had become a notably pale orange tone. By Day 7, the eggs had turned yellow, which, in turn, had been transformed into a brown coloration by Day 10. Finally, on Day 13, the coloration of the eggs was observed as grayish.

The gravid females were measured and weighed, with the first embryonic observation undertaken in order to establish the stage of development of the egg. The segmentation was

Once the larvae had hatched from the eggs, volumetric tests were carried out to calculate the number of hatchings, following the procedure described in [25], manual, in which ten samples of the homogeneous mixture are taken from the tank in a 100 ml cup. All the larvae identified in each sample were then counted, after which the total number of larvae observed was multiplied by the number of liters in the tank and divided by the total number of liters comprising the samples, which corresponded to one liter. This procedure was applied for all the experimental units. Reproductive success is expressed via the relationship between the rates of embryo production and hatching [19], wherein the hatching percentage was obtained by dividing the number of larvae observed for each treatment by the total number of larvae hatched. The survival rate was obtained using the following formula, as proposed by [32]:

$$Survival (\%) (S) = 100 - (or g . beginning - or g . end / or g . beginning) \times 100.$$

### 3. Results

The ovigerous females ranged from 10.5 cm to 14 cm in length, giving an average  $12.3 \pm 1$ , which was also within the length range for females without embryos. In terms of weight, the ovigerous females ranged from 18 g to 38 g, with an average of  $24.8 \pm 5.4$  g, while their final weight corresponded to a minimum of 17 g and a maximum of 34 g, with an average of  $22.8 \pm 4.8$  g (Table 1).

observed as complete and the surface of the egg appeared lighter than its center, which can be explained by the fact that gastrulation was beginning, thus developing the embryonic primordium. Seventy-two hours later, the metanauplius was observed to have recently appeared, with a transparent trunk (caudal papilla), small reddish chromatophore spots, and ocular pigmentation (optic lobe) of a darker tone also observed, while the appendages were barely visible. The postnaupliar stage had presented by Day 7, with the appendages lengthening and becoming larger, while the

caudal papilla was observed to be larger and in the shape of the letter C. moreover, the telson was now presenting, the chromatophores were developing in more parts of the embryo, the dorsal cardiac vessel was now formed and beating and the eyes were completely differentiated. By Day 10, the embryo had increased considerably in size, its movements increasing as the size of the vitellus decreased, with abdominal segments forming in the trunk, the eyes pedunculated, the heart developing, and the chromatophores now clearly distinguishable.

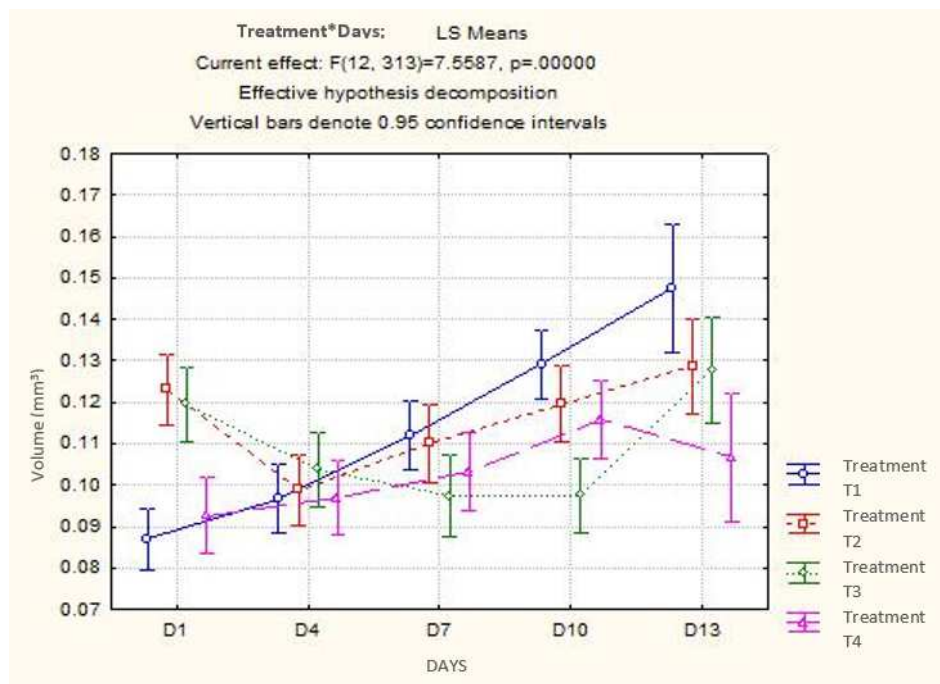
Finally, by day 13, the larva was fully formed and ready to be introduced into the medium, with the embryo occupying all available space except for the remaining dorsal yolk, while all the thoracic and cephalic appendages covered the ventral side of the egg and the extremities of both the appendages and the telson presented setae. At this point, the eyes were observed to grow larger, while the embryos had finished feeding on the yolk, thus occupying even more space

in the egg.

On Day 1, treatments 1 and 4 began with a lower egg volume (0.08 and 0.09 mm<sup>3</sup>, respectively) than treatments 2 and 3 (0.12 and 0.11 mm<sup>3</sup>, respectively), which had presented a higher initial volume, differences which are significant ( $F = 18.262$ ;  $p < 0.01$ ). By Day 4, the volume of the embryos did not present significant differences ( $F = 0.913$ ;  $p = 0.438997$ ), a variable which was still presenting no significant changes by Day 7 ( $F = 2.133$ ;  $p = 0.104672$ ). By Day 10, the volume of the embryos continued to increase for all treatments, except Treatment 3, in which the embryo remained the same size, a contrast which was found to be statistically significant ( $F = 7.951$ ;  $p = 0.000126$ ). By Day 13, the embryos were presenting continuous volume increases, except for Treatment 4, in which a decrease in volume was observed. However, the foregoing differences were not found to be statistically significant ( $F = 2.481$ ;  $p = 0.081328$ ) (Figure 1).

**Table 2.** Average number of *M. rosenbergii* hatches observed for the different salinity treatments, corresponding to the number of treatment days.

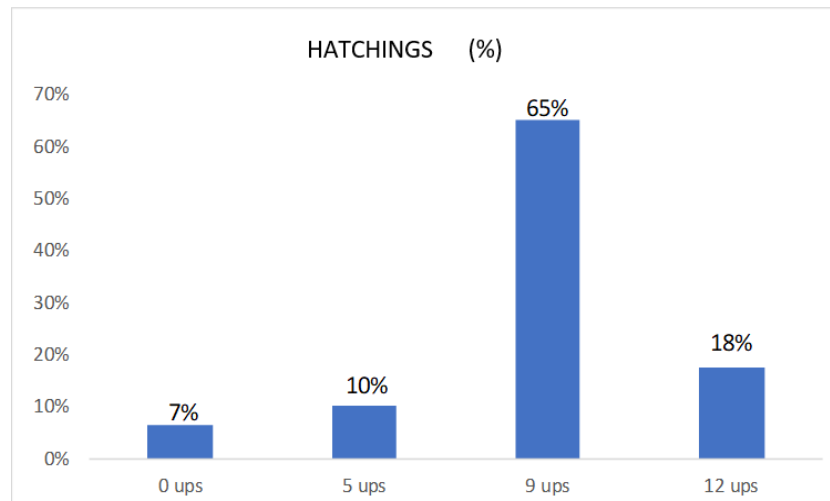
Day	T1 (0 ups)	T2 (5 ups)	T3 (9 ups)	T4 (12 ups)
1	0.876 ( $\pm 0.0046$ )	0.1245 ( $\pm 0.0128$ )	0.1195 ( $\pm 0.0104$ )	0.0922 ( $\pm 0.0173$ )
4	0.0971 ( $\pm 0.0067$ )	0.0981 ( $\pm 0.0075$ )	0.1020 ( $\pm 0.0182$ )	0.0956 ( $\pm 0.0145$ )
7	0.1121 ( $\pm 0.0066$ )	0.1095 ( $\pm 0.0143$ )	0.0974 ( $\pm 0.0194$ )	0.1042 ( $\pm 0.0234$ )
10	0.1338 ( $\pm 0.0216$ )	0.1199 ( $\pm 0.0088$ )	0.0975 ( $\pm 0.0215$ )	0.1170 ( $\pm 0.0199$ )
13	0.1475 ( $\pm 0.0081$ )	0.1300 ( $\pm 0.0203$ )	0.1303 ( $\pm 0.0326$ )	0.1068 ( $\pm 0.0359$ )
	0.1156	0.1164	0.1093	0.1032



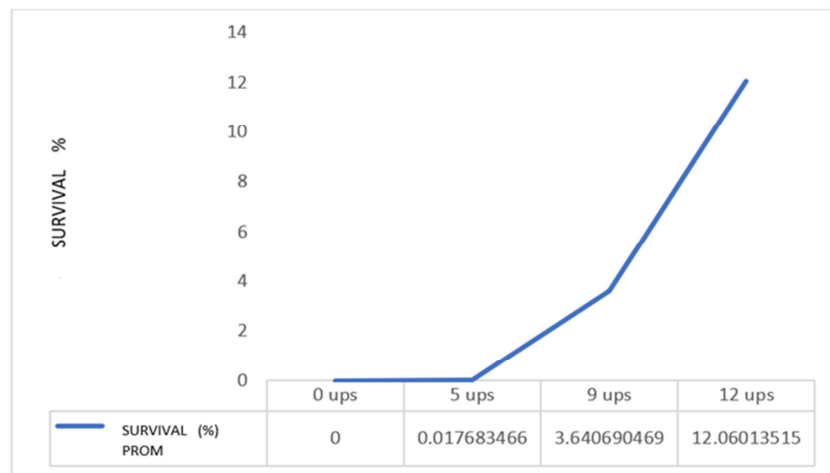
**Figure 1.** *M. rosenbergii* embryo volume corresponding to the number of days comprising each treatment.

The highest number of hatchings was observed in Treatment 3, at a salinity of 9 psu, followed by Treatment 4 (12 psu), for which a hatch percentage of 18% was observed, while the lowest values for this variable were observed for treatments 1 and 2 (0 and 5 psu, respectively) (Table 2).

The highest percentage of hatchings was observed for Treatment 3 at a salinity of 9 psu, followed by Treatment 4 (12 psu) with 18%, while the lowest percentages were observed for treatments 1 and 2 (0 and 5 psu, respectively) (Figure 2):



**Figure 2.** (%) of *M. rosenbergii* hatchings obtained for the different salinity treatments.



**Figure 3.** Percentage of survival according to the degree of salinity.

Once the embryo had fully developed, the total number of hatchings for each treatment was calculated, after which the zoea larvae were kept alive until Stage 11 of embryonic development, at which point the experiment was concluded. For each salinity treatment to which *M. rosenbergii* was subject in the present study, it was observed that the survival percentage tends to increase as the salinities increase, rising from zero or null for Treatment 1 (0 psu) to 0.017% for Treatment 2 (5 psu), 3.64% for Treatment 3 (9 psu) and 12.06% for Treatment 4 (12 psu) (Figure 3).

## 4. Discussion

Physicochemical factors play an important role in the development of organisms, especially in the early stages of life [4]. [26] found that both the commercial quality and growth of *Metapenaeus bennettiae* juveniles is clearly influenced by factors such as temperature and salinity from the organism's first life processes onwards.

In the present study, embryonic development comprised 13 days for all treatments, in contrast to the control treatment (0 psu), wherein one of the replicas completed their

development 24 hours later. [10], reported a total embryonic duration of 24 days, subjecting the palaemonid *Cryphiops caementarius* to different salinity treatments at a temperature of  $22.5 \pm 1.5$  ° C, a finding which contrasts to the results reported for *Macrobrachium*. [4] reported an embryonic development of 20 days, identifying a trend where, as salinity increases, the hatching time for *M. rosenbergii* embryos reduces, a finding which contrasts with that obtained by the present study. While there are similarities between the two studies in terms of species and treatments, there are also significant differences, with [4], applying a temperature of  $27 \pm 1$  ° C and the present study applying an average temperature of  $29.5$  ° C. The foregoing also corresponds to the findings obtained by [13], who found that the embryonic development of *M. amazonicum* lasted 12 to 15 days at  $30 \pm 1$  ° C and 19 to 24 days at  $24 \pm 1$  ° C. The morphological characteristics observed in the present study corresponded to those reported by [13], with the one difference that the eggs obtained for Treatment 1 (0 psu) in the present research tended to become more fragile to handling as the days passed. The foregoing contrasts with [1], who observed the conclusion of the embryonic development of *M. americanum*

after 17 days at 28.1°C (a different temperature than that applied in the present research), clearly indicating, at a species level, different durations for the first stages of life of these organisms.

Reported an embryonic duration of 20 days for *M. rosenbergii* and observed various egg mass tones: bright orange; yellow; pale brown; and, dark brown. [14] In contrast to that found by the present research, they observed five different colorations, the first two of which are similar to those that we observed, while the last two were different, with the penultimate a brownish yellow and the last tending to be paler. Similarly, [1], described three types of coloration in *M. americanum* eggs over the course of embryonic development, starting with bright orange and followed by light brown and, finally, gray. This finding is explained by the increasing size of the eyes and the fact that, as the embryos finish feeding on the yolk, they acquire a higher proportion of space in the egg, as described by [7] and [22]. This difference in egg coloration is probably due to the different geographical locations in which the above described studies were carried out [4].

A constantly increasing egg volume was observed for Treatment 1 (0 psu) of the present study, rising from 0.0876 to 0.1475 mm, with this treatment observed to have produced the highest volume by the end of the study. This finding contrasts with [10], wherein, despite similar volume increases being observed for *C. caementarius* in freshwater (0.0665–0.1170 mm<sup>3</sup>), the last embryonic stage saw a greater volume increase in eggs incubated at 10 and 20 psu (0.0640 - 0.1192 and 0.0669 - 0.1201 mm<sup>3</sup>, respectively). They also describe a phenomenon similar to that found in the present study, where, while greater volume was obtained, shrinkage was observed at Stage 5 (Day 7) of embryonic development, which was then followed by further increases in volume. Said differences in volumetric behavior in the eggs may be due to the differences between the genera studied. The eggs obtained from the control treatment (0 psu) in the present study were larger than those obtained from the different salinity a treatment, which also concurs with [28], who reported that the size of *M. malcolmsonii* eggs tends to decrease as salinity increases. This may be due to osmotic stress, expressed via cell shrinkage at higher salinities. [2], report that osmotic tolerance in crustaceans depends on the embryo's external membrane inside the egg, the semi-permeability of which decreases when the embryonic membranes are being formed and then increases when they are fully formed. They also observed a tendency for lower egg volume as salinity increases, due to the fact that the eggs gradually become larger. The embryos produced by Treatment 1 (0 psu) in the present study tended to swell, enabling the intracellular passage of more solvents and, thus, causing the egg to swell, which does not necessarily occur due to nutritional intake or to the benefit of the quality of the embryo. On the last day of the present experiment, Day 13, a trend can be observed in the eggs, wherein those produced by Treatment 1 were the largest (swollen by freshwater), followed by Treatment 2 with a size of 5 psu, which, in turn, is slightly larger than those produced via Treatment 3, with 9 psu. The

eggs produced via Treatment 4 (12 psu), which corresponded to the highest salinity of all the treatments, presented the lowest volume. This finding may be related to the presence of more ions in the passage of the external medium into the embryo, as reported by [8], who also report 100% mortality in *M. americanum* larvae at Stage 5 of embryonic development at 0 psu in green water. This finding differs with data obtained for *M. rosenbergii* in the present study, wherein 100% mortality was observed in larvae at Stage 2 (Treatment 1), which may be due to the fact that the study used clear water.

A tendency for a higher hatching percentage was observed for the higher salinity treatments than for the lower salinity treatments, which contrasts with that reported by [10], who found, in a study conducted on the embryonic development of *C. caementarius*, a higher hatching percentage at 0 psu than at both 10 and 20 psu. The foregoing contrast may indicate that *C. caementarius* reproduces in freshwater without problems, unlike the development of *M. rosenbergii* from embryogenesis onwards.

The survival percentage revealed by the present study shows a trend that is directly proportional to increased salinity, with Treatment 4 found to achieve the best rates of larvae survival once they have left the confines of the egg. The above finding concurs with that reported by [4], who, having applied six different treatments (0, 6, 9, 12, 16, and 18 psu), reported an increasing survival rate (as a percentage) up to the 12 psu treatment, with said rate then tending to decrease for the last two treatments. They also describe 12 psu as the best salinity of the levels studied [25], thus indicating salinity as a key factor for the larval development of *M. rosenbergii*.

The survival rate observed for Treatment 1 (0 psu) shows that hatching does not, in itself, guarantee survival, with the 100% mortality observed at Stage 2 of embryonic development, as described by [6]. This indicates that, while embryos tolerate increased water levels, larvae necessarily require saltwater in order for metamorphosis to occur. It is impossible for larvae to continue developing without saltwater, as [1] describe for *M. americanum*, finding 100% mortality at 1 psu salinity, with no survivors observed by Day 2 of said treatment, and reporting almost 100% survival, up to Stage 3, for all the other treatments (12, 16, 20, 24, and 26 psu) applied.

## 5. Conclusions

The conditions presented by an organism are a subsequent representation of the success or failure of the culture medium in which it has been grown. It is important to take into account the fact that the factors affecting success or failure vary depending on the geographical location in which the research is undertaken. The present research considered various factors in order to obtain a culture of high quality, ensuring that the development of the *M. rosenbergii* embryo occurred over practically the same hatching time, thus acquiring similar morphological features. A slight change in structure can be seen in eggs obtained at 0 psu (Treatment 1),

where they are more fragile when handled and tend to break more easily, due to the amount of water they contain, thus causing the solvent to enter the egg in greater quantity and resulting in a greater volume. Moreover, the present study reveals the influence of temperature on embryonic development, which accelerates as the temperature increases (29.5°C), while higher volumes do not necessarily entail optimal organisms, as the higher the salinity level, the higher the number of expected hatchings. However, the results of the present study show that Treatment 3, at 9 psu, obtained the highest hatching percentage (65%), which was significantly higher than that obtained via Treatment 4, at 12 psu (18%), which indicates a good level of salinity, but in a measured proportion to embryonic development. Once the embryos had hatched, the larvae showed the foregoing salinity preference, wherein it was observed that, as salinity increased, survival also increased, with, of the salinity treatments applied, 12 psu (12.06%) found to be the optimal level for larval development.

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