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Salinity effect on embryonic development and survival of the first zoeal stage of *Macrobrachium tenellum* (Smith, 1871) (Crustacea, Palaemonidae)

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Abstract. The objective of this work is to provide information to understand the effect of different salinities on the survival of embryos and newly-hatched larvae of *Macrobrachium tenellum*. The survival of *M. tenellum* embryos was similar in all treatments up to nine days with an average of 94%. At day 12, survival was higher at the lower salinities (90 and 80% at 10 and 0 psu, respectively), whereas total mortality was observed at 30 psu. There were no significant differences in treatments 10 and 0 psu. The treatment that showed the higher hatching percentage was 10 psu (82%); hatching at 0 and 20 psu treatments were 60 and 55%, respectively. Larvae kept at 10 psu showed higher survival 7 days after hatching (98%), meanwhile, larvae maintained in freshwater (0 psu) failed to survive beyond the fifth day (keeping the larvae without food). According to the results obtained in this research, embryonic development of *M. tenellun* can develop between 0 and 10 psu, whereas, optimal salinity for hatching and development of first larval stages are between 10 and 9 psu.

Key words: prawn, embryo, larvae, *in vitro*, hatching

Resumen: Efecto de la salinidad en el desarrollo embrionario y la supervivencia del primer estadio zoea de *Macrobrachium tenellum* (Crustacea, Palaemonidae). El objetivo de este trabajo es proveer información que ayude a comprender el efecto de diferentes salinidades en la supervivencia de embriones y larvas recién eclosionadas de *Macrobrachium tenellum.* La supervivencia de los embriones de *M tenellum* fue similar en todos los tratamientos hasta los nueve días con un promedio de 94%. A los 12 días, la supervivencia fue de 0% a 30 ups, mientras que en los demás tratamientos (20, 10 y 0 ups) fue de 50, 90 y 80 % respectivamente. No se encontraron diferencias significativas en los tratamientos 10 y 0 ups. El tratamiento que mostró mayor porcentaje de eclosión fue 10 ups (82%) en comparación con los tratamientos de 0 y 20 ups donde eclosionaron el 60 y 55% respectivamente. En 10 ups las larvas mostraron la supervivencia más alta a los 7 días posteriores a la eclosión (98%), por el contrario las larvas mantenidas en agua dulce (0 ups) no lograron sobrevivir más allá del quinto día (manteniendo las larvas sin alimento). De acuerdo con los resultados obtenidos en esta investigación, el desarrollo embrionario de *M. tenellun* puede desarrollarse entre 0 y 10 ups, mientras que la

salinidad óptima para la eclosión y el desarrollo de las primeras etapas larvarias están entre 10 y 9 ups.

Palabras clave: langostino, embrión, larvas, *in vitro*, eclosión.

Introduction

The colonization of crustaceans of freshwater environments has been possible due to certain physiological adjustments that include sophisticated mechanisms for the transportation of passive and active ions and the decrease in the permeability of the exoskeleton (Intanai *et al*. 2009). These physiological capabilities vary depending on species, their life history and the life stage. In the particular case of the genus *Macrobrachium*, these adaptations are important because the species are distributed in environments where variations in salinity may be important (Garcia-Guerrero *et al*. 2013). Moreover, their larvae require brackish water for their survival and development (Bueno & Rodrigues, 1995). There are different osmoregulators patterns in larvae and adults that can vary by species according to the different degrees of adaptation to life in freshwater (Anger 2001). Prawns of the genus *Macrobrachium* have a hemolymph with high osmolarity in freshwater and tolerate a wide range of salinities (Ordiano *et al*. 2005). The understanding of this phenomenon is particularly important in larvae as their osmotic needs may be completely different from that of adults and vary even between different larval stages. Anger (2001) mentioned that salinity is one of the environmental parameters with more influence in aquatic animals, particularly those living in estuaries, due to the wide variation of salinity. Salinity tolerance is characteristic of each species and seems to be related to the ecology (Anger, 2003) and its isosmotic point (Moreira *et al*., 1983).

Macrobrachium tenellum is a decapod crustacean of the Palaemonidae family with amphidromy behavior as almost all species of the genus (Signoret-Poillon & Soto 1997). It is a resource used by the inhabitants of the coast as a direct and indirect source of food in almost all its geographical distribution area, Baja California, Mexico (27 ° N) to the Chira River, Peru (5 ° S) (García-Guerrero *et al*., 2013). In the Mexican Pacific slope, this species, together with *M. americanum*, contributes more than 3,000 t annually to the national market (Pérez-Velázquez *et al*., 2011) and its demand increases year after year at the local and regional levels. However, the lack of studies on its larviculture does not allow it to be scaled to commercial culture levels.

The species reproduces during the entire year with peaks in summer (at the end of the rainy season). At that time, river flows increase near the coastal outfall where salinity is approximate to 12 psu (Vega-Villasante *et al*. 2011). Due to the fact that adults can stay in brackish water they can be found either in estuaries, rivers and coastal lagoons (Guzmán 1977, Román-Contreras, 1991), showing a good capacity of osmoregulation (Alpuche *et al*. 2005), which allows them to adapt to high salinities during dry season (Chung 2001).

Eventhough adult prawns of *M. tenellum* can tolerate wide ranges of salinity from 0 to 30 psu (Signoret-Poillon & Soto 1997, Vega-Villasante *et al*. 2011), the effect of different salinities on survival and growth rates has not been well studied yet. Vega-Villasante *et al*. (2011) showed that adult prawns are able to grow at salinities higher than 25 psu, obtaining better growth in salinities of 0 to 10 psu. However, it is unknown the effect of different salinity concentrations on larval survival of *M. tenellum*, and larval osmoregulatory capacity is much more limited than in adults (Greenwood *et al*., 1989, Bas & Spivak, 2000, Charmantier & Charmantier-Daures, 2001). The lack of knowledge about the osmoregulatory capacities of *M. tenellum* larvae implies an obstacle to develop their culture. For this reason, the objective of this work was to provide information on the effect of different salinities on survival of embryos and newly hatched larvae of *M. tenellum.*

Materials and Methods

To determine the effect of different saline concentrations in embryos of *M. tenellum*, a female was selected and the ovigerous mass (embryonic Stage I: newly spawned eggs) (Wehrtmann, 1990) was extracted for in *vitro culture.* Four salinities were evaluated with three replicates each (0, 10, 20 and 30 psu). Salinity concentrations were selected according to conditions previously observed in the wild (unpublished data) where ovigerous females were found (with the exception of 30 psu). EU consisted of 12 flasks of 50 mL adjusted to 30 mL. The different concentrations of salinity were adjusted from marine water filtered at 0.45 microns

and diluted with purified water. The EU were placed in an incubator (PRECISION®) at a constant temperature (29 ± 1 °C) and were provided with oxygen through the movement of a stirring plate (CIMAREC®). To each treatment, methylene blue at a concentration of 2 mL/L was supplied to prevent bacterial and fungal infections. Every third day the entire water was replace and the EU was changed with flasks previously sterilized, with the same concentrations of salinity and methylene blue. Embryos were observed each 48 h under a microscope (AMSCOPE®), and dead embryos were removed and accounted. The percentage of hatching was determined by counting the hatched larvae. Newly-hatched larvae were maintained without feed (at the same conditions of embryos) and survival was recorded every 24 h.

To determine the effect of different saline concentrations in zoeas of *M. tenellum*, ten ovigerous females from the Ameca River (Jalisco-Nayarit, México. 20° 40′ 21″ N, 105° 16′ 51.6″ W) and carrying embryos in stages II and III **(**Wehrtmann, 1990) were collected and transferred to the Laboratorio de Calidad de Agua y Acuicultura Experimental of Universidad de Guadalajara. They were kept individually within freshwater in plastic containers (40 L) provided with constant aeration and shelters. They were fed daily with commercial pellet feed for marine shrimp (Purina® 35% protein). After hatching (6 to 8 hours), larvae were transferred directly to the experimental units. All larvae used came from a single female. The different concentrations of salinity (0, 3, 6, 9, 12 and 15 psu) were adjusted with purified freshwater and commercial salts (RED SEA®), according to our previous observations (F. Vega-Villasante, unpublished data) of larvae in the wild. The experimental units (EU) consisted of 100 ml white opaque cylindrical plastic containers. Ten larvae were placed in each EU. The treatments had six replications. During the study period (five days) larvae were kept without feed, under a natural photoperiod for the area (12:12), and at a temperature of 28 ± 2 °C. The behavior (atypical swimming, location in the water column) (Villalon, 1991) and larval survival was recorded on a daily basis. The larval mortality was confirmed when the larvae did not present movements at all after a direct tactile stimulus, and removed immediately. When necessary, water lost by evaporation was replaced with freshwater adjusting to the corresponding salinity.

Comparisons were made with parametric and non-parametric statistical procedures. When the data did not meet these assumptions (normality and homogeneity of variance), they were log or arc-sin transformed. If transformed data did not meet the assumptions, a Kruskal-Wallis test was performed. Differences between survival rates at different salinities, as well as differences in hatching percentage were tested using one-way ANOVA. When the ANOVA was significant, differences between treatments were tested *a posteriori* with a Tukey ($\alpha = 0.05$) test.

Results

The survival of *M. tenellum* embryos was similar in all treatments up to the ninth days and was on average 94% ($P > 0.05$). At day 11, embryonic survival was statistically similar in 0 and 10 psu treatments (90 and 95% respectively) ($F = 44.9$, d.f. = 3, $P < 0.001$). At 12 day, the survival of the embryos showed statistical differences between treatments ($F = 64.9$, d.f. = 3, $P < 0.001$) and was similar in 0 and 10 ups (80 and 90% respectively) and lower in the 20 psu trial (55 %); however, there were no significant differences between 20 and 0 psu ($P > 0.05$). At 30 psu all embryos died at day 12 (Fig. 1).

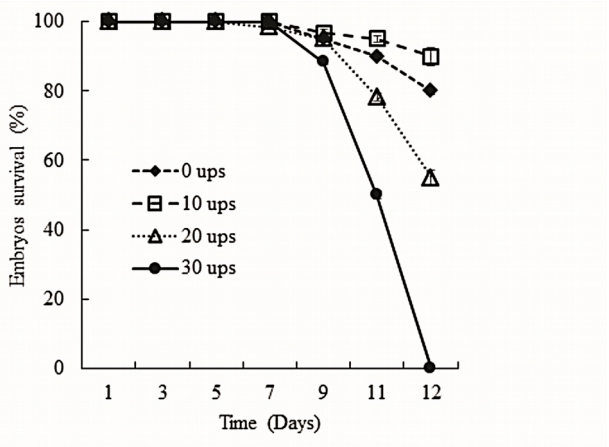

Figure 1. Survival (%) of embryos of *Macrobrachium tenellum* cultivated at different salinities. Figurneeds to be improved.

The embryos of *M. tenellum* started hatching after 12 days at 0, 10 and 20 psu. Hatching rates differed significantly among salinities ($F = 5.05$, d.f. = 2, $P = 0.01$) (Fig. 2).

Larvae hatched in the 10 psu treatment showed higher survival at 7 days after hatching (98%)compared to larvae maintained in freshwater (0 psu) which failed to survive beyond the fifth day

(keeping the larvae without feed) (Fig. 3). In the 10 psu treatment, larvae molted to second zoeal stage within 3-4 days after hatching.

There was no mortality at the moment of transferring the larvae directly from the 0 psu salinity to the treatments. In all treatments except for 0 psu, the zoeae I were swimming actively on the surface or in the water column. The percentage of larvae survival of *M. tenellum* maintained at different salinities in relation to the exposure time is presented in Figure 4. Statistical analysis revealed significant survival differences during the first 24 hours of exposure in the treatment with 0 psu ($H = 11.58$, d.f. = 5, $P < 0.05$), on the contrary to the other treatments (3, 6, 9, 12, 15 psu) which responded in a statistically similar way. Final survival of the 9 psu treatment (28.3%) was significantly higher ($H = 16.19$, d.f. = 5, $P = 0.006$).

Discussion

Among the environmental parameters, salinity plays a crucial role for development, hatching and survival of embryos and larvae of species of the genus *Macrobrachium* (Jayalakshmy & Natarajan 1996, Anger 2001). Our results showed that the embryonic development of *M. tenellum* could be achieved successfully from spawning to hatching in a range of salinity from 0 to 10 psu. However, according to the different salinity concentrations evaluated in this study (0, 10, 20 and 30 psu) a significant effect between treatments was demonstrated ($P < 0.05$) in the survival of the embryos. In this sense, Damrongphol *et al.* (2001) reported that optimal concentrations of NaCl (7 psu) were essential for the development, survival and hatching of embryos of *M. rosenbergii*; in addition, variations in the levels of NaCl or KCl (169.2mM of NaCl and 3.6 mM KCl) drastically altered embryonic development. In our study, the longest survival of embryos was obtained at 0 and 10 psu (80 and 90%, respectively).

The embryos of *M. tenellum* exposed to 30 psu probably died of dehydration (Fuentes *et al*. 2010). Similar results reported Hangsapreurke *et al*. (2008), who described that the best salinity range was between 5 and 15 psu for *M. rosenbergii.* For the same species (*M. rosenbergii*), New (1990) reported an optimum salinity for the incubation of equal or less than 15 psu. Soundarapandian (2008) mentioned that in *M. malcolmsonii* the incubation period was shortest at salinities of 7 psu, without finding significant differences for 0.5, 3.0, 10.5 and 14.0 psu treatments. Similarly, Ituarte *et al*. (2005)

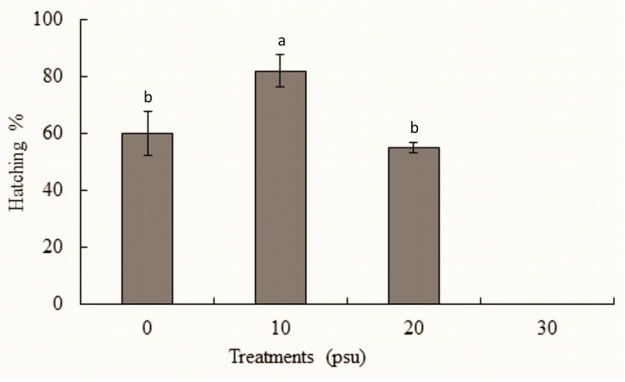

Figure 2. Percentage of hatching of embryos of *Macrobrachium tenellum* cultivated at different salinities.

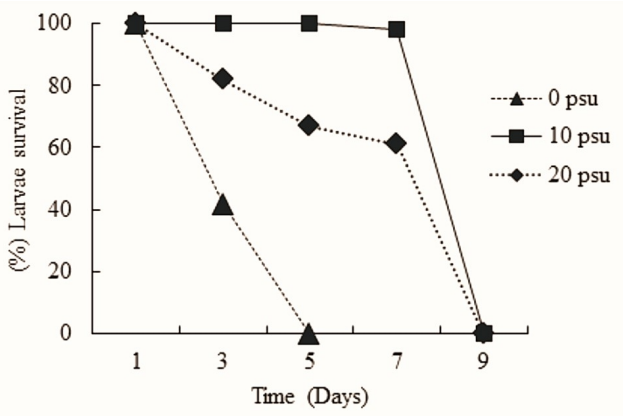

Figure 3. Survival (%) of larvae of *Macrobrachium tenellum* cultivated at different salinities and without fees.

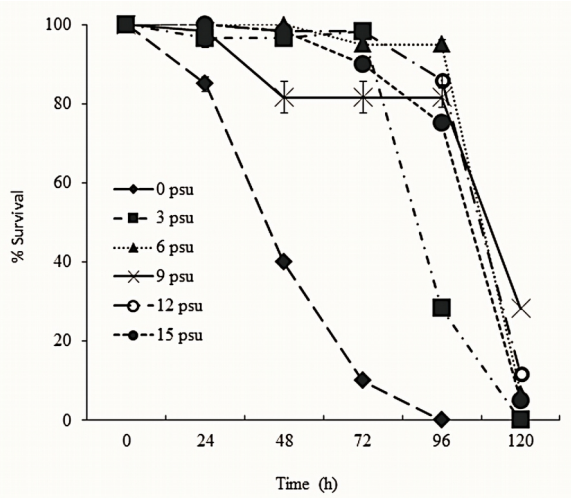

Figure 4. Survival (%) as a function of salinity in the first zoea of *Macrobrachium tenellum*.

reported for *Palaemonetes argentinus*, the best embryo survival at concentrations of 1 and 15 psu. However, for embryos of *Cryphiops caementarius* a salinity of 10 psu (or higher), generated a lower survival, and caused deformations of the larvae (Fuentes *et al*. 2010), which suggested that salinity

tolerance or osmoregulatory capacity is characteristic of each species.

The percentage of hatched embryos of *M. tenellum* obtained at 10 psu (82%) incubated in vitro was similar to results of other studies with species of the same genus: Soundarapandian (2008) working with *M. malcolmsonii* reported a percentage of hatching of 94% at 7 psu. Damrongphol *et al.* (1990) mentioned that salinity of 5.25 and 10.5 psu (artificial sea water) provided an optimal media for the development of embryos and hatching in *M. rosenbergii*. With the same specie Habashy & Hassan (2011) evaluated three treatments (0, 8 and 16 psu) and indicated that the highest percentage of hatching was obtained at a salinity of 8 psu at 29 °C. Ratnayake *et al*. (2011) and Caluwe *et al*. (1995) reported that the best salinity for hatching in *M. rosenbergii* was 6 psu, while optimal salinity for hatching in *M. idella* was 5 psu (Jayalakshmy & Natarajan 1996).

The null hatching of embryos of *M. tenellum* at the highest salinity concentration tested in this study was possibly caused by a passive loss of water through the egg membrane at an early stage of embryonic development (Charmantier & Charmantier-Daures 2001). In the 30 psu treatment, the water loss could be higher compared to low-salinity treatments, which possibly provoked an egg shrinking, causing irreversible physiological damages to the embryo (Ituarte *et al*. 2005). This effect was less pronounced, suggesting not caused irreversible damage in the 20 psu treatment (some hatching was recorded at this concentration). According to Ismael & New (2000), naturally hatching of *M. rosenbergii* larvae occurs under estuarine conditions and is higher in brackish water than in freshwater.

Adult aquatic crustaceans have adaptations such as high permeability and good intra- and extracellular osmotic capacity to avoid osmotic stress (Charmantier 1998). The larvae, however, are more vulnerable due the poor development of these physiological abilities (Anger 2001). They are very small and they are unable to move freely in the water column. This exposes them to osmotic stress, which often involves mortal osmotic shock (Anger, 2003). Also, the larvae have different capabilities to adapt to physical and chemical changes in the water compared to adults and juveniles , therefore, the evolution has favored its develop in environments where the salinity is closer to its isosmotic point (Anger 2001). Because of this, many species of *Macrobrachium*, females or larvae, perform reproductive migrations into brackish water zones, environments with the most appropriate physicochemical water characteristics for larval development (Bauer 2013). As a consequence, its tolerance to physicochemical changes in the water also varies, particularly salinity tolerance that frequently varies more (in coastal and estuarine waters), which can generate a negative effect on the organism (Anger 2001, Torres *et al*. 2007). Our results coincide with the aforementioned reports as the best larval survival were recorded in the 9 and 12 psu treatments. In relation to the above, Anger and Hyde (2009) showed that at 10 psu, larvae of *M. amazonicum* survive up to 14 days without external feed intake. The above suggests that these animals were kept in salinities close to their isosmotic point and, therefore, very similar to its optimum salinity (≈ 10 psu, Vega-Villasante *et al.* 2011) and due to this with greater possibilities of survival (Souza *et al*. 1997). These results coincide also with those from Cabrera *et al*. (1979) who kept successfully larvae of *M. tenellum* from hatching to metamorphosis in a salinity of 12 psu. Subramanian *et al*. (1980) mentioned that the best salinities for the survival of larvae of *M. idea* were between 5 and 15 psu. For larvae of *M. rosenbergii*, Ling & Costello (1979) reported salinities of 12 to 15 psu and Ra ´anan & Cohen (1982) of 12 psu. Chung (2001) indicated that the highest survival for larvae of *M. carcinus* was at salinities between 5 and 15 psu. On the other hand, Moreira *et al*. (1983) obtained the curves of respiratory metabolism of the first zoeae from several species of *Macrobrachium* at different salinities and observed two different trends: The first group included *M. acanthurus* and *M. olfersii* and showed a higher oxygen consumption at low and medium salinity concentrations. The second group, which included *M. heterochirus* and *M. carcinus*, showed a decrease in the oxygen consumption in low and high salinities. Apparently, *M. tenellum* would be in the first group as other studies suggested that in freshwater the metabolic rate is reduced by 38% (Aguilar *et al*. 1998). This hyper-regulatory behavior in low salinities (0-20 psu) (Signoret-Poillon & Soto 1997) and hypoconformer in high salinities is characteristic a euryhaline organism (Aguilar-Juarez 1995, Vega-Villasante *et al*. 2011).

The present results showed that all larvae died within 96 h when maintained in freshwater. There are several possible aspects of this phenomenon, which may have caused this high mortality in freshwater. According to Intanai *et al*. (2009) the respiratory rate and protein synthesis are the first

affected processes when the salinity is not suitable. The same author suggests that salinity affects adults or postlarvae in different way, being a more drastic effect in the last ones as the capacity to synthesize or degrade proteins, or exchange ions and metabolites are particularly crucial in these stages. And, at least for *M. rosenbergii*, the isosmotic point does not change with the life cycle stage, but each stage has different capacities to cope with salinity changes. When there is too much energy directed for osmoregulation, the organism may die. Similar results were reported by Ismael & Moreira (1997) for zoeae of *M. acanthurus*, which did not survive more than 5-6 days in freshwater. Similarly, Subramanian *et al*. (1980) registered low larval survival (5%) of *M. idea* in freshwater. It is possible that the larvae of *M. tenellum* do not die immediately in freshwater due to their status as an amphidromous species, which confers some resistance to this adverse situation (Vargas-Ceballos, 2018). Bauer (2011) mentioned that in the amphidromous prawns, females spawn in rivers where also the larvae hatch to migrate quickly with the help of the river currents and reach areas with a higher concentration of salinity. In another scenario, gravid females migrate downstream, with the aim of carrying eggs as closely to brackish conditions as possible. In this sense whichever the scenario is, larvae should be able to tolerate temporarily freshwater conditions before arriving areas with salinity conditions required to complete their development. In case they do not reach these places within a time limit, mortality would occur.

In the present work, zoeae I of *M. tenellum* survived a period of 3 to 4 days in freshwater. Read (1985a) stated that in their natural environment zoeae of *M. petersi* remained overnight close to the water surface (where the flow rate is higher than at the bottom) (Read 1985b) and transported the larvae down the river to brackish water. Such a strategy and considering the ability to survive for several days in freshwater favor that the larvae reach optimal salinities for their development. It is important to consider, however, that salinity is only one of multiple factors that should be optimal to favor larval survive and growth.

According to the results obtained in this research, embryonic development of *M. tenellun* can be achieved in salinities between 0 and 10 psu, whereas optimal salinity for hatching and development of first larval stages are between 10 and 9 psu respectively. These results provide relevant data to better understand its life cycle and consequently valuable information for the development of adequate culture technology. Additional studies are needed to provide new insights of other physico-chemical attributes of the water or the environment that are also involved.

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