

Research Article

Experimental culture of the river prawn *Macrobrachium americanum* larvae (Bate, 1868), with emphasis on feeding and stocking density effect on survival

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ABSTRACT. The cauque river prawn *Macrobrachium americanum* occurs along the Pacific coast of America. This prawn can grow to a large size, making it an interesting option for aquaculture production. Currently, supplies of juveniles are limited because hatchery and laboratory-reared larvae are difficult to raise. This study assesses larval survival for different combinations of stocking density and feeding from larvae cultivated in green water. From these combinations, larvae fed with *Artemia* nauplii and maintained at a density of 50 larvae L⁻¹ had the highest survival.

Keywords: *Macrobrachium americanum*, larval development, feeding, survival, prawn.

Cultivo experimental de larvas de langostino *Macrobrachium americanum* (Bate, 1868), con énfasis en alimentación y efecto de la densidad sobre la supervivencia

RESUMEN. El langostino de río *Macrobrachium americanum*, ocurre a lo largo de la costa Pacífica de América. Este langostino puede alcanzar grandes tallas lo que lo convierte en una opción interesante para la acuicultura. En la actualidad, el abastecimiento de juveniles a partir del cultivo de sus larvas en hatchery y en laboratorio son difíciles de criar. Este estudio evalúa la supervivencia de las larvas con diferentes combinaciones de alimento y densidades de población cultivadas en agua verde. A partir de estas combinaciones, las larvas alimentadas con nauplios de *Artemia salina*, mantenidas a una densidad de 50 larvas L⁻¹, tuvieron la mayor supervivencia.

Palabras clave: *Macrobrachium americanum*, desarrollo larvario, alimentación, supervivencia, langostino.

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INTRODUCTION

There are more than 220 species of freshwater prawns in the genus *Macrobrachium* (Bate, 1868) (New, 2002). Some species, such as *M. americanum* or *M. carcinus*, reach large sizes, which make them attractive to fisheries. Because of overexploitation,

pollution, and human impact on rivers and shorelines, their populations have declined in Latin America (Mantellato & Barbosa, 2005). In Mexico, protective regulations have been developed (NOM-009-PESC-1993; Diario Oficial de la Federación, Mexico City, August 2006). Exotic species, such as *M. rosenbergii*, the only species of this genus whose production relies

on aquaculture, may cause ecological problems (Yan *et al.*, 2001). Cultivation of native species may provide good yields when grown in their place of origin, and production does not affect habitat conservation. For example, repopulation or insertion of laboratory-reared juveniles can be placed in locations where prawns are disappearing because of overfishing, pollution, or habitat destruction. Additionally, diversification of fisheries products, based on farming of native species could reduce the environmental impact of crustacean cultivation and could better fit with local markets (Vega-Villasante *et al.*, 2011). *Macrobrachium americanum* is one species that has potential for aquaculture, yet has not been sufficiently studied.

This prawn is found in rivers of the Pacific coast of the Americas, from Isla Cedros (Baja California, Mexico) to Paita, Perú (Hendrickx, 1995). Local fisheries have severely diminished the natural populations, yet there is a lack of field studies that may provide information about the current status of its populations. Local fishermen observed that with each passing year, fewer prawns appeared in traps and nets. Some studies suggested its productive potential (García-Guerrero, 2009, 2010; García-Guerrero *et al.*, 2011), because *M. americanum* is similar to prawns that are currently cultivated. Unfortunately, production of larvae is a major bottleneck because appropriate larval production techniques have not been developed. Success in larval production in the laboratory is the main goal before production or conservation purposes can be seriously pursued. Field and laboratory studies are needed to identify ideal larval conditions, specifically nutritional and water quality requirements. Previous studies found that *Macrobrachium* larvae need live and fresh food to survive, mainly *Artemia* nauplii or fresh ingredients, such as fish meet or egg custard (Araujo & Valenti, 2007). For *M. americanum*, Monaco (1975) is the only study offering preliminary results on larval rearing, suggesting the utilization of fresh food in low salinity water. The purpose of the present study is to determine whether some low stocking densities in combination with food and water quality may result in higher survival rates for larvae cultivation.

MATERIALS AND METHODS

General procedure

M. americanum (Bate, 1868) adults were caught in Río Coyuca (17°03'36.14"N, 108°27'42.78"W) in Coyuca de Benítez, Guerrero, Mexico, with traps, by net, and by hand. Only specimens with all legs, including berried females, were collected. They were

transported and cultivated as described by García-Guerrero & Apún-Molina (2008). Berried females were placed in individual 50 L plastic tray tanks and eggs were monitored. Hatching was verified as described by García-Guerrero & Hendrickx (2009). At 48 h before hatching, females were transferred into 150 L plastic tanks filled with freshwater at 28°C and continuous aeration to maintain dissolved oxygen levels $>6 \text{ mg L}^{-1}$. Most larvae hatched at night; the next morning, they were collected with a siphon, aided by their positive response to light, and transferred to 50 L experimental replica tanks with continuous aeration. Larvae were counted after mixing the water by gentle movement with a paddle. Then a 50 mL sample was taken and all larvae from this sample were counted five times to determine density. Water in experimental tanks was filtered (5 μm) and passed through a UV lamp. To prevent diseases, parasites or bad water quality, water was treated with the following chemicals: EDTA (0.5 mg L^{-1} ; Scelzo, 1998), iodine (dissolved at 1.75%), calcium hydroxide (20 mg L^{-1} ; Mallo & Fenucci, 2004), copper sulfate (125 mg L^{-1} ; Scelzo, 1997), formaldehyde (1×10^{-5} ppt; Hernández-Valencia, 2010) and sulfamethoxazole-trimethoprim (4×10^{-2} ppt; 23,750 mg +1,250 mg; Laganà *et al.*, 2011). A 5 hp blower (Sweetwater®, Apopka, FL, USA) and diffusers were used to maintain continuous aeration. Temperature was monitored daily and maintained at $28 \pm 0.5^\circ\text{C}$; pH was measured weekly with a pH meter (American Marine®, Ridgefield, CT, USA), and maintained at 8.2 ± 0.1 . Daily water changes consisted in 20% water volume in each tank. Hypersaline water was used to raise salinity, which was set at 6 ppt during the first 24 h and then varied in agreement with Table 1 (Hernández-Valencia, 2010). Dissolved oxygen was monitored daily (YSI 55 oxymeter, YSI, Yellow Spring, OH) and maintained at levels above 7.0 mg L^{-1} . Natural photoperiod was 14 light and 10 dark, without direct sunlight. Green water was prepared in a 16 m^3 concrete outdoor tank filled with fresh water. This was a mixture of *Chaetoceros* spp. (Ehrenberg), *Chlorella* sp. (Beijerinck), and *Isochrysis galbana* (Parke). This culture was managed as described by Chu (1989) and Bray *et al.* (1990), and maintained at a concentration of $4 \times 10^5 \text{ cells mL}^{-1}$ (Hernández-Valencia, 2010). The concentration of the culture was measured daily. Larval stages were determined following the criteria of Choudhury (1970, 1971).

Before starting an experiment, some assays were performed. Those assays were made in succession, so the positive results in one influenced the next one. The first assay determined if females should be collected from the wild with recently-produced eggs or eggs close to hatching, based on egg clutch appearance and

Table 1. Relationship between cultivation day and salinity utilized during the larval culture of river prawn *Macrobrachium americanum*.

Day	Salinity
0	6
1-2	8 -10
3-4-5	12, 14, 16
6-8	17, 18, 18
9-10	19-20
11-13	20
14-15	21
16	23
17-18	25
19-20	25
21-22	26
23-24	27
25-28	28
29-30	28
31-32	27
33	26
34-37	25
38-44	25
45-84	25
49-84	25

hatching rate. Several females were captured with eggs at initial or late developmental stages, as described by García-Guerrero & Hendrickx (2009). Only larvae coming from females collected with eggs close to hatching were included in subsequent assays, because females with recently-produced eggs developed infections. This problem was mostly caused by fungi and partial infections of egg masses, so the hatching rate was lower.

The second assay was designed to determine the proper food. Four different meals were prepared, similar to what is normally used for other *Macrobrachium* larvae (Monaco, 1975; Daniels *et al.*, 1992; New, 2002; Araujo & Valenti, 2007). Larvae were stocked at a density of 50 L⁻¹ in water with microalgae maintained at a concentration of 4×10⁵ cells mL⁻¹. The following diets were used: D₁ (dry and pulverized white fish *Chirostoma* sp. of Mexico); D₂ (recently-hatched *Artemia* nauplii; D₃ (live zooplankton (several live copepods, mixed, and unidentified) collected in a shrimp farm at Coyuca de Benitez, Gro., Mexico); D₄ (fresh bonito *Sarda* sp. meat. Larvae and microalgae density were measured every day. Food was given at fixed times and rations: 07:00 (30%), 10:00 (20%), 12:00 (20%), 14:00 (15%), and 18:00 (15%). Since *Artemia* nauplii were easier to

manage, larvae were fed with recently-hatched and refrigerated *Artemia* nauplii in further assays.

The third assay was based on microalgae combinations (green water) in the experimental units. *Chaetoceros* sp. and green water treatments were prepared and maintained at 12 ppt. *Chaetoceros* sp. was grown in 200 L fiberglass indoor columns, while green water was prepared in a 16 m³ outdoor tank. Larvae were stocked at a density of 50 L⁻¹. After hatching, *Artemia* nauplii were refrigerated for one hour; then fed to the larvae (three nauplii per mL every day starting with the second day). Nauplii (were previously disinfected according to the instructions provided by the supplier MS-222, Argent Chemical Laboratories, Redmond, WA). In the green water culture, larvae survived three days more in comparison with *Chaetoceros* water; therefore, green water was used in further experiments.

Based on these results, an experiment was designed to test larval stocking densities among treatments. Low densities were chosen to avoid cannibalism, which is common in this genus (Barros & Valenti, 2003). Densities were: 10 (T₁), 20 (T₂), 30 (T₃), 40 (T₄), and 50 (T₅) larvae per liter. Nauplii were provided at 07:00, 10:00, 13:00 and 16:00 h, and the water tested to maintain a concentration of 3 nauplii per mL. Green water was maintained in all treatments at a concentration of 4×10⁵ cells L⁻¹. Salinity in the tanks was adjusted as presented in Table 1.

Statistical analysis

The means for survival were calculated for all treatments. Subsequently, means were compared by ANOVA and when differences were present, a further comparison was made with Fisher's Least Significant Difference (LSD test, P < 0.05). The means of ∞ survival (%) over time showed two trends; therefore two models were used to describe them. The first model (-μt + 100) was defined as mortality index (μ). This linear model was valid from 0 ... t₀, in which the last value corresponds to the intersection point. The second model was described as an exponential model: Ae^{-α(t-t₀)} in which A is survival estimated at time t₀, e is the base log and α is the exponential decay rate and is defined as morbidity. The criteria used to determine the parameters of the models imply that the area below the curve described by the mean of survival of each treatment is equal to the area below the curve of the model. Linear trends in survival, which depends on different densities during the first week (acclimation time), was analyzed with a quadratic model (Shearer, 2000). Percentage of survival for this time period was calculated as:

$$\#L_{PA(0-1)} \cdot 100\% L \cdot \#L_{ini}^{-1}$$

in which 100% L is the percentage of larvae at stocking; $\#L_{PA(0-1)}$ is the amount of larvae at the end of the first week and $\#L_{ini}$ is the amount of larvae at stocking. Differences in survival between treatments were determined at the end of the trial.

RESULTS

No differences in larval survival were observed among the experiments with different food types (Fig. 1). However, *Artemia* nauplii were better in terms of water transparency and odor. This diet was easier to manage and had better acceptance by the larvae, which was determined by the existence of nauplii traces in larval digestive tracts.

The formal experiment lasted 12 weeks, at the end of which at least 100 postlarvae were obtained. Duration and day of appearance of larval stages are presented in Table 2. In agreement with the regression formulae of mortality per treatment, differences in survival denote that the lowest survival rate was obtained in T₂ (Fig. 2); however, there were no statistical differences when comparing the slopes between treatments for the entire trial ($P < 0.05$). The highest correlation with mortality was between T₂ and T₃ ($r^2 = 0.999$), while the lowest correlation was between T₁ and T₅ ($r^2 = 0.840$). Treatment T₁ had higher mortality compared to the others, at least until week 6 ($\alpha_{(0-12)} = 0.35$), followed by T₃ and T₄ (both with $\alpha_{(7-12)} = 0.70$). Treatment T₂ and T₅ had mortality slopes of ($\alpha_{(7-12)} = 0.87$) and ($\alpha_{(7-12)} = 0.71$), respectively. Mean survival during acclimation (morbidity) between treatments was compared ($P < 0.05$), and statistical differences are presented in Figure 3, which shows that survival directly increases with density.

DISCUSSION

During egg development, the egg mass on berried females of *M. americanum* could get infected by fungi or parasites (García-Guerrero, 2009; García-Guerrero & Hendrickx, 2009). In the wild, females clean and groom the eggs and remove those which are dead or infected (García-Guerrero, 2009). The stress from captivity may inhibit this behavior (Graziani *et al.*, 1993), and this could be a cause for egg mortality. The green water technique is often used for marine shrimp larval culture because it provides less direct light and nitrogen waste (Nunes *et al.*, 2011).

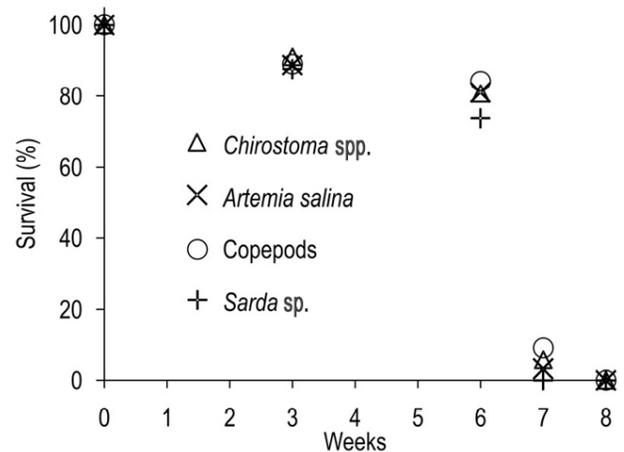


Figure 1. Survival during experimental culture of river prawn *Macrobrachium americanum* larvae fed with different types of food.

Table 2. Days of apparition of developmental stages during the larval development of river prawn *Macrobrachium americanum* during the culture.

Day	Stage
0	I
1-2	II
3-4-5	III
6-8	IV
9-10	V
11-18	VI
17-18	VI, VI
19-23	VII
24-28	VIII
29-37	IX
38-44	X
45-84	XI
49-84	I, PL

Some food treatments do not seem suitable for larval culture since most of the dead larvae had empty or almost empty digestive tracts. Fresh fish utilized as food in the feeding trials was rarely eaten by the larvae. Such meals are hard to turn into particles that are less than 100 μm . The larger particles quickly lose freshness and contaminate the water (Monaco, 1975). Additionally, there is no evidence that dried and pulverized fish was eaten by larvae in the Monaco study. Copepods seem to be the food which less deteriorates water quality, but were hard to obtain and have no proven nutritional value. *Artemia* nauplii were too large for early larval stages. We refrigerated *Artemia* nauplii to reduce movement, but early larvae did not have the capacity to catch them. Monaco

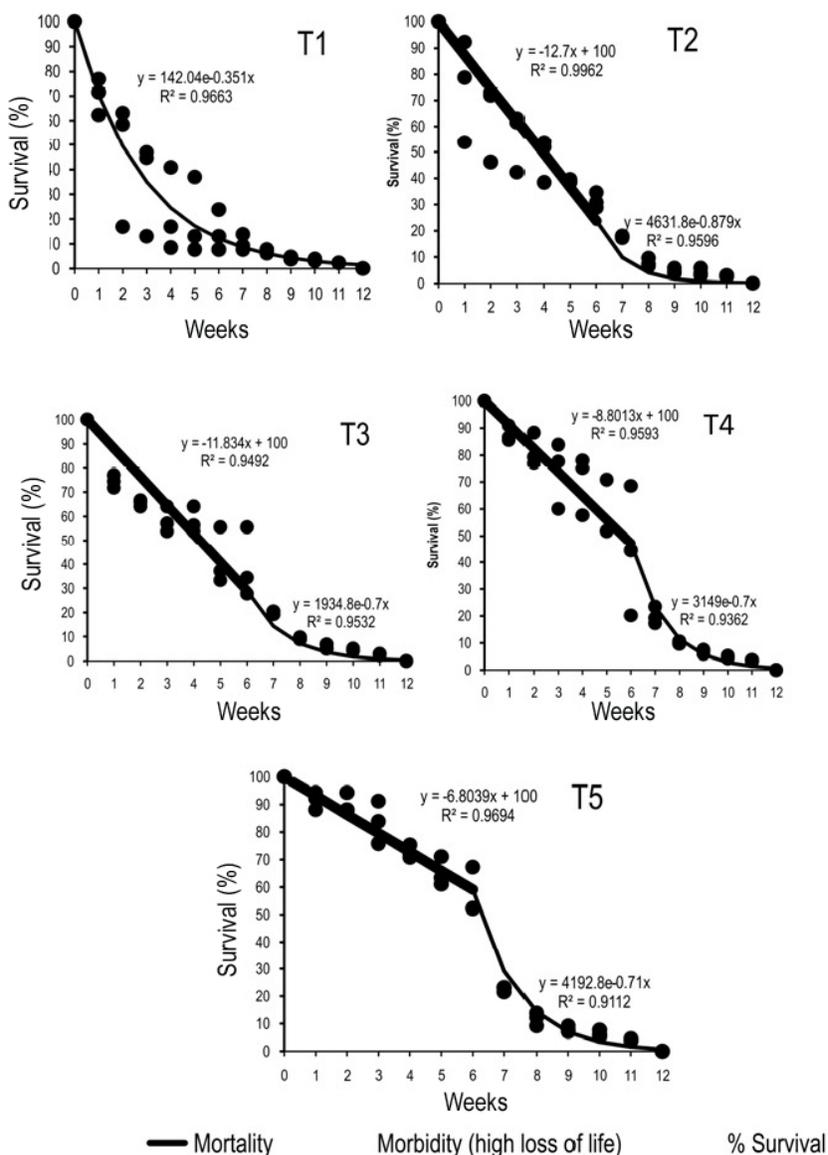


Figure 2. Survival during experimental culture of river prawn *Macrobrachium americanum* larvae stocked at different densities. Bilinear adjustments of the five treatments, where: wide line: Mortality, slim line: Morbidity (big loss of life), black spots: original values (means) of percentage of survival. T₁ = 10 larvae L⁻¹; T₂ = 20 larvae L⁻¹; T₃ = 30 larvae L⁻¹; T₄ = 40 larvae L⁻¹; T₅ = 10 larvae L⁻¹.

(1975), used *Artemia* nauplii together with *Chlamydomonas* to feed larvae of this species, but with little success. It is unknown if a diet of *Artemia* nauplii meets the nutritional requirements for these larvae. The use of *Artemia* is common in the larval culture of *M. rosenbergii* (Barros & Valenti, 2003; Velu & Munuswamy, 2008), but these larvae are larger than *M. americanum* larvae. In general, feeding problems were the main cause for low survival. We assume that larvae in our experiment did not have sufficient energy to successfully molt to the next stage due to a

lack of nutrition. Although the results of our experiments does not offer conclusions about proper nutrition, previous studies showed that feeding is one of the main keys to larval survival (García-Guerrero & Apún-Molina, 2008; García-Guerrero, 2010), which in fact is defined as the principal index to determine success or failure of larval cultivation (Daniels *et al.*, 1992).

From previous research with prawn larvae (Anger, 2001; Anger *et al.*, 2009), it is known that physiological and anatomical differences between stages

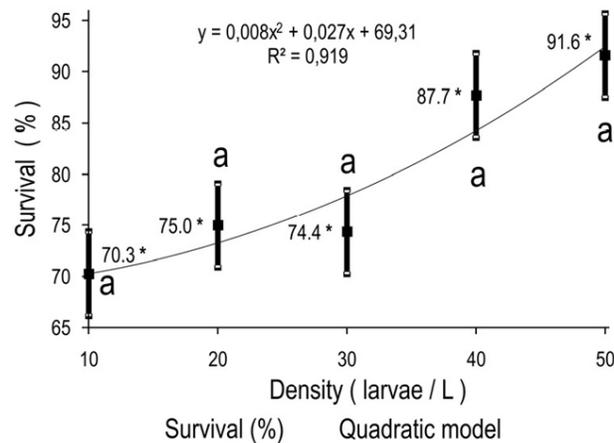


Figure 3. Percent of survival (\pm standard error) at the end of the acclimation week (first week) for river prawn *Macrobrachium americanum* larvae stocked at different densities. There were no statistical differences ($P > 0.05$) when comparing the survival percentages among treatments for the acclimatization week. (*) This symbol indicates the existence of correlations ($P > 0.05$) among treatment values.

affect survival, particularly their ability to eat during the first three stages, when physiological readjustments require additional energy (Agard, 1999). Recently-hatched larvae still have yolk, which enables them to survive one or two molts without feeding (García-Guerrero & Hendrick, 2009). Afterwards, larvae must feed (Choudhury, 1970; Monaco, 1975), and actively search for food particles that are suitable and easy to catch (Persoone & Sorgeloos, 1980). Correia *et al.* (2000), showed that *M. rosenbergii* larvae become more voracious from Stage VII onwards and *Artemia* nauplii is routinely used as a food source for larvae. However, if larvae did not feed properly in previous stages, they ultimately die since they are too weak to pursue any kind of prey. This is a point-of-no-return, the moment when larvae had lost their chance for survival (see Anger, 2001).

Our results also showed that density is the second most important cause of larval death. Differences in μ values after the first week indicated that higher densities provided better survival during cultivation. It seems that mortality during the first week is related to low stocking density because the highest mortality occurred at the lowest stocking density, 10 larvae L^{-1} (29.7%, $\mu PA_{(\text{week } 0-1)} = -29.744$). The highest survival occurred at 50 larvae L^{-1} (8.4%, $\mu PA_{(\text{week } 0-1)} = -8.4013$). Morbidity occurred after the first week. Studies using different stocking densities showed significant differences in survival; higher survival occurred at lower stocking density, but those studies

used *M. rosenbergii*, which has larger larvae at hatching and has well established larval cultivation techniques (100 larvae L^{-1} *Macrobrachium rosenbergii*; Nhan *et al.*, 2009), showing that density heavily affects larval survival at high stockings (Graça-Melo & Brossi-Garcia, 2005). Nhan *et al.* (2009), found that densities over 200 are critical since tolerance to stress and starvation decreases at higher densities. According to these authors, recommendable densities are about 100 larvae L^{-1} ; low densities imply more available nauplii per larvae, but also more surplus nauplii in the culture which negatively affects water quality and has a negative effect on survival (Lavens *et al.*, 2000). Barros & Valenti (2003), noticed that high densities of nauplii encouraged larvae to consume more food. Prawn hatcheries commonly use larval-rearing densities ranging from 40-60 larvae L^{-1} in green water systems and 80-100 larvae L^{-1} in open and recirculation systems (Phuong *et al.*, 2006). All these findings apply to *M. rosenbergii*, where well-developed production techniques exist.

In conclusion, suitable, small live food is one of the main concerns for raising *M. americanum* larvae. To improve survival, and considering the results of our study, a density of 50-200 larvae L^{-1} is recommended, together with live food smaller than *Artemia* nauplii, such as some freshwater rotifers, which has been suggested by several authors (Lovett & Felder, 1988; Lubzens *et al.*, 1989).

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