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Short Communication

Intake of different food sources in the first zoeae stages of *Macrobrachium tenellum* (Decapoda: Palaemonidae)

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ABSTRACT. The objective of this work was to assess the acceptance of live and inert food by *Macrobrachium tenellum* during the early larval stages. The larvae were obtained by collecting wild ovigerous females in the Ameca River in the State of Jalisco, Mexico. Eight treatments (diets) were used to feed the larvae: D1, control (fasting); D2, micro-pulverized food (Purina®); D3, living, newly hatched nauplii of *Artemia franciscana* (INVE®, Salt Lake City, Utah, USA); D4, commercial paste containing microalgae (Instant Algae Rotifer Diet®); D5, water extracted from a biofloc system; D6, cooked egg yolk; D7, newly hatched and frozen nauplii of *A. franciscana* (INVE®, Salt Lake City, Utah, USA); D8, nutritional supplement for shrimp larvae (Epifeed LH1®). Treatments D2, D5, D6 and D8 showed traces of food in the digestive system. The larvae did not consume D3 and D7 treatments. The diets that had more acceptance were micro-pulverized food, a nutritional supplement for shrimp larvae Epifeed® LH1, cooked egg yolk, and biofloc water.

Keywords: prawn; zoea; *Artemia*; food intake; inert food; nutrition; aquaculture

Aquaculture is one of the fastest-growing economic activities worldwide, requiring constant improvements in the management of different aquatic species. Among crustaceans, the genus *Macrobrachium* (Bate, 1868) is comprised of 243 species of freshwater prawns (De Grave & Fransen, 2011), of which only some have proven to be of interest for aquaculture (García-Guerrero *et al*., 2013). Nowadays, the global demand for prawns is mainly met by the Asian species *M. rosenbergii*, which is widely cultivated around the world (New, 2009). However, this species is listed as exotic, which means that it might cause ecological damage if introduced to countries outside of its natural range of distribution (Yan *et al*., 2001). The use of native species in aquaculture has several advantages: the animals adapt more quickly to the environment, the environmental impact of aquaculture activities is minimized, and the product has better acceptance in local markets (Yamasaki-Granados *et al*., 2013).

Macrobrachium tenellum is a species native to Mexico of high commercial interest; however, the scarcity of studies on the larval stages of this species has not allowed cultivating it for commercial purposes. Nutritional factors are among the most important factors in the larval development of decapod crustaceans such as prawns. The larval stage, which is the most critical developmental stage for crustaceans, is when they start feeding exogenously, after the absorption of the yolk sac (Luna-Figueroa *et al*., 2010). However, knowledge about the digestive processes and nutritional requirements of prawn larvae is very limited (Araujo & Valenti, 2007). According to Barros & Valenti (2003b), larvae of different species of *Macrobrachium* show different stages of development,

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different morphology, behavior, and nutritional needs; furthermore, they generally have small buccal openings and a small yolk reserve. They need an exogenous food source in the first few days after hatching. The main source of live food in commercial crustacean larviculture is newly hatched *Artemia* nauplii (Barros & Valenti, 2003a). The use of this type of live food for prawn larval feeding has several advantages, including easy handling and high content of essential nutrients (Lavens *et al*., 2000). However, some authors have reported that *Artemia* nauplii do not provide the nutrients required during the last larval stages of *M. rosenbergii*, and thus recommend the use of dietary supplements (Valenti *et al*., 1998; Valenti & Daniels, 2000; Barros & Valenti, 2003a).

Moreover, some crustacean species are not strictly carnivores during all larval stages (Araujo & Valenti, 2007). For these reasons, some authors have suggested the use of alternative food sources (live and inert) as a complement or replacement of *Artemia* nauplii in crustacean larviculture (Alam *et al*., 1995a,b; Silva & Rodriguez, 1997; Barros & Valenti, 2003a). The objective of the present study was to assess the intake of live and inert food by *M. tenellum* in the early larval stages.

The study was carried out in the Laboratorio de Calidad de Agua y Acuicultura Experimental of Universidad de Guadalajara (LACUIC), located at the Centro Universitario de la Costa in Puerto Vallarta, Jalisco, Mexico. The larvae were obtained by collecting wild ovigerous females in the Ameca River, Jalisco, Mexico. Ovigerous females carrying stage III eggs (Wehrtmann, 1990) were selected and separated individually into experimental units of 40 L, with water adjusted to salinity of 10, an average temperature of 29.0 ± 0.5°C and constant aeration; they were fed with a commercial feed with 35% protein and 8% fat (Purina®). After the embryos hatched, the larvae were removed and placed in reservoirs with the same characteristics of salinity and temperature as before. Eight treatments (diets) were used to feed the larvae: D1, control (fasting); D2, micro-pulverized (<200 µm) feed for marine shrimp (48% crude protein) (Purina®); D3, newly hatched, living nauplii of *Artemia franciscana* (INVE®, Salt Lake City, Utah, USA); D4, commercial microalgae concentrate (Instant Algae Rotifer Diet®); D5, water extracted from a biofloc system; D6, cooked egg yolk; D7, frozen, newly hatched nauplii of *A. franciscana* (INVE®, Salt Lake City, Utah, USA); D8, inert diet for marine shrimp larvae (Epifeed LHF1®). The production of nauplii of *A. franciscana* was performed according to Vega-Villasante *et al.* (2013). For the assays to measure food intake, 24 plastic containers (150 mL capacity) were used, each with 100 mL of water adjusted to salinity of 10, previously prepared by mixing marine and purified freshwater. Ten larvae were used (collected one day after hatching, zoea I) per experimental unit. Each treatment was performed in triplicate. The larvae were deprived of food for 2 h and then fed once with different treatments. After 30 min, all larvae were collected, put in a Petri dish, and examined under a stereomicroscope to determine the presence of food particles inside the digestive tract. The digestive content was classified as follows: a) absence of food (both the stomach and the intestine were empty); b) the presence of food (the stomach and the intestine were full or partially full). The acceptability (%) of the treatments was determined by the ratio between the number of larvae with the presence of food in the stomach or intestine, and the total number of larvae observed. One-way ANOVA was used to compare the data obtained in this experiment (after verifying normality and homoscedasticity of variances), followed by Tukey's test. The significance level was set at $P < 0.05$. All the analyses were performed using the software "SigmaPlot" (version 11.0).

Table 1. Food detected in the different areas of the digestive tract of zoea II larvae of *Macrobrachium tenellum.* X represents the presence of food particles. D1: control (fasting); D2: micro-pulverized food (Purina®); D3: newly hatched, living nauplii of *Artemia franciscana* (INVE®, Salt Lake City, Utah, USA); D4: commercial concentrate of microalgae (Instant algae®); D5: water extracted from a biofloc system; D6: cooked egg yolk; D7: newly hatched and frozen nauplii of *A. franciscana.* (INVE®, Salt Lake City, Utah, USA); D8: inert diet for shrimp larvae (Epifeed LH1®).

Treatments	Stomach	Intestine
D1	-	-
D2	X	X
D3	-	-
D4	-	-
D5	X	
D6	X	
D7	-	-
D8	X	X

Table 1 shows the results obtained by feeding larvae of *M. tenellum* with the different diets evaluated. Treatments D2, D5, D6 and D8 showed traces of food in some sections of the digestive tract (Fig. 1). Treatments D2 and D8 showed traces of food in the stomach and the intestine in the first 30 min of exposure. Treatments D5 and D6 showed food particles only in the stomach. Treatments D3, D4 and D7 were not consumed by the larvae (zoea II). The percentage of acceptance differed significantly for each treatment

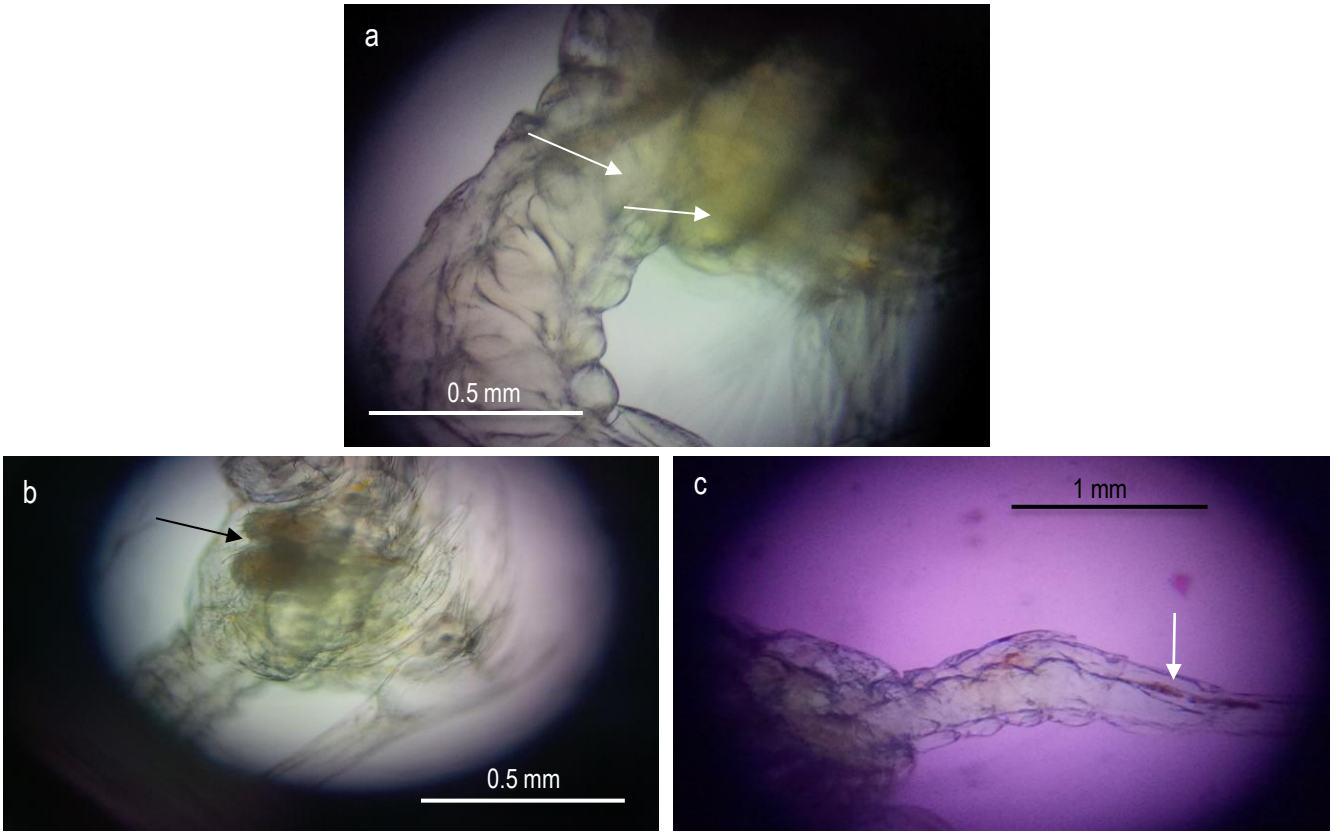

Figure 1. Food consumed by zoea II larvae of *Macrobrachium tenellum:* a) hepatopancreas and the first portion of the hindgut with commercial micro-pulverized food, b) hepatopancreas with Epifeed LHI® food, c) final portion of the hindgut with Epifeed LHI® particles (the arrows in every case show the mentioned area).

(ANOVA, F = 6.78, d.l. = 3, P = 0.01) (considering only the treatments that had some degree of acceptance) (Fig. 2). Treatments D2, D5 and D8 (93.3, 76.7, 80%) were statistically similar. Treatment D6 (43.3%) was significantly different from D2 (Tukey, P = 0.01) and D5 from D8 (Tukey, P > 0.05).

The larval stage is one of the most critical in the culture of prawns because, after consuming the yolk reserves, feeding becomes a priority. Anger & Hayd (2009) reported that during the initial phase of larval development, the larvae use their fat reserves not only in the absence of food but also when enough food is available, although in the latter case, they use their fat reserves more slowly. Programmed lipid degradation ensures that maternal energy, previously invested in embryos, is eventually converted into metabolic energy and in the precursors that are necessary for the synthesis of new tissues. Some prawn species, such as *M. amazonicum* and *M. rosenbergii*, can feed on other organisms (carnivorous behavior) from the second day after consuming their yolk sac (Barros & Valenti, 2003a; Araujo & Valenti, 2007). The use of different

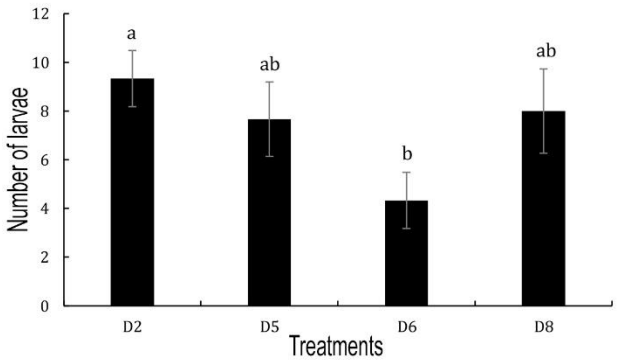

Figure 2. The number of *Macrobrachium tenellum* larvae that had food remains in some sections of the digestive system (in the accepted diets). The values of the treatments are presented in triplicate, mean ± standard deviation. Different letters indicate significant differences (P > 0.05).

nutritional sources by *M. rosenbergii* during the larval stage (zoea II) has already have been studied, but the larvae of other species do not consume the same type of diet (Lavens *et al.*, 2000). Anger & Hayd (2009) re-

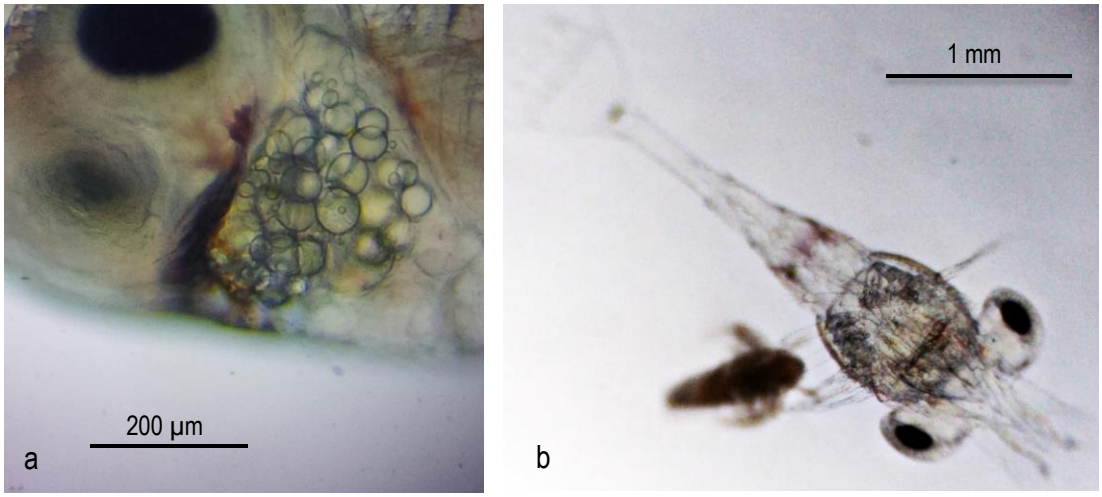

Figure 3. a) Lipid supplies of zoea I of *Macrobrachium tenellum*, b) *Artemia franciscana* nauplii and zoea II of *Macrobrachium tenellum.*

ported that in the early larval stages, Palaemonidae shrimps, including some marine species, often feed partially on plankton, while other shrimps pass through lecithotrophic phases during facultative stages.

In the present study, the zoea I stage did not consume food, regardless of the treatment. This feeding behavior has been mentioned by other authors who reported that the zoea I of *M. rosenbergii* and *M. amazonicum* are lecithotrophic and survive on yolk reserves during the first days (Barros & Valenti, 1997; Araujo & Valenti, 2007). The zoea I of *M. tenellum* was found to have some lipid reserves in the cephalothoracic region that allows it to grow into the next stage without exogenous feeding (Fig. 1). The first record of the presence of food in the intestine of *M. tenellum* larvae occurred in the zoea II stage, as digested food particles were observed moving from the hepatopancreas towards the hindgut (Fig. 3) which agrees with the reports of Barros & Valenti (2003a) and Anger & Hayd (2009), who determined that the early stages of larval development of *M. rosenbergii* and *M. amazonicum* depend little on exogenous food sources, even though zoea II shows intermittent lecithotrophic behavior, being able to accept or reject exogenous feeding. Of the evaluated treatments, the ones that showed greater acceptance (traces of food in any section of the digestive tract) by zoea II were D2, D5, D6 and D8. Except for treatment D5, the treatments that were accepted by the larvae consisted of inert food.

However, inert diets were found to be too inefficient, mainly due to the lack of knowledge about the nutritional needs of the larvae (Sorgeloos & Léger, 1992). Barros & Valenti (2003a) mentioned that, in inert diets, the size of food particles, their consistency, texture and density could influence their acceptability to larvae. These same authors reported that the level of the water column could affect the frequency of food intake in zoea II of *M. rosenbergii*, mainly by allowing particles to disperse better and remain in suspension for more extended periods, increasing the probability of being caught and eaten by larvae. Currently, in addition to providing *Artemia* nauplii throughout the larval cycle, most commercial hatcheries of *M. rosenbergii* provide a wet diet known as egg flan (Levens *et al*., 2000). In the present study, the egg yolk treatment was accepted by larvae of *M. tenellum.*

The larvae fed with water extracted from the biofloc system (D5 treatment) had some food particles in the digestive tract. It is known that water from a mature biofloc culture will probably contain rotifers (Loureiro *et al*., 2012) and nematodes (Ray *et al*., 2010) as well as bacteria and yeasts (Monroy-Dosta *et al*., 2013). Barros & Valenti (2003a) suggested that penaeid larvae have a good rate of survival and acceptable development when cultured with rotifers. Silva & Rodriguez (1997) concluded that in the larviculture of *M. rosenbergii*, 34% of *Artemia* nauplii could be replaced with the nematode *Panagrellus redivivus* from larval stage V, and up to 66% after stage VII.

This experimental essay verified that the early larval stages of *M. tenellum* (zoea II) could not eat *A. franciscana* nauplii due to their size, as it is practically impossible for larvae to capture the nauplii and feed on them. However, *Artemia* nauplii constitute the main live food source in commercial crustacean larviculture (Barros & Valenti, 2003a) and are commonly used in the larviculture of *M. rosenbergii* (Levens *et al*., 2000; Barros & Valenti, 2003b; Nhan *et al*., 2010). Similarly,

Gomes *et al.* (2014) mentioned that it is possible to feed the postlarvae of *M. equids* feed with *Artemia* nauplii. It is worth noting that the first larvae of *M. rosenbergii* (2.2 mm) (Levens *et al*., 2000) and *M. equidens* (1.9-2 mm) (Ngoc-Ho, 1976) are larger than the zoea I of *M. tenellum* (1.5-1.7 mm).

In the present study, *Artemia* nauplii were frozen in order to stop their motility and then supplied to larvae of *M. tenellum*, but they were not able to eat them. Similar results were found by Yamasaki-Granados *et al*. (2013), who reported that *M. americanum* failed to feed on frozen *Artemia* nauplii. Feeding *M. tenellum* larvae with newly hatched nauplii of *Artemia* did not work, as has been reported for other *Macrobrachium* species, at least during the first larval stages (zoea I and zoea II). Micro pulverized food, water from a biofloc system, and Epifeed LH1® represents an alternative for feeding *M. tenellum* larvae, at least in their early stages. However, further study is required.

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