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Feeding and larval growth of an exotic freshwater prawn *Macrobrachium equidens* (Decapoda: Palaemonidae), from Northeastern Pará, Amazon Region

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ABSTRACT

In the present study, we carried out experiments on the diet of the freshwater prawn *Macrobrachium equidens*. We tested which type of food and which density of food is suitable for larval development. For the experiment on the type of food, eight treatments were carried out: (I) starvation, (AL) microalgae, (RO) rotifers, (AN) *Artemia,* (RO + AN) rotifers + *Artemia,* (AL + RO) microalgae + rotifers, (AL + AN) microalgae + *Artemia*, (AL + RO + AN) microalgae + rotifers + *Artemia*. For the experiment on the density of food, we used the type of food, which had resulted in a high survival rate in the previous experiment. Three treatments were carried out: 4, 8 and 16 *Artemia* nauplii /mL. The rate of feeding during larval development was observed. The survival, weight and percentage of juveniles of each feeding experiment were determined. We found that larvae are carnivores; however, they have requirements with respect to the type of food, because larvae completed their cycle from the zoeal to the juvenile stage only when *Artemia* nauplii were available. We also verified that the larvae feed mainly during the day-time, and are opportunistic with respect to the density of food offered.

Key words: Palaemonidae, diet, food preference, live food.

INTRODUCTION

Recent studies have revealed several specimens of *Macrobrachium*, which are morphologically different from individuals of the same species recorded for the region of Brazil. The analysis of the mitochondrial cytochrome oxidase subunit I gene (COI) has shown that their sequences diverge 20% from those of *M. acanthurus* (a native species) and from other sympatric, native species. When the sequences of these individuals were compared with sequences in the GenBank database, we found that they were actually *Macrobrachium equidens* (Dana 1852), an exotic species native to the Indo-Pacific region (Maciel et al. 2011).

As *M. equidens* (Dana 1852) is considered an exotic prawn species to the region of Brazil, studies that aim to increase knowledge on this species, including biology, interactions with other congeneric, native species of the same genus, and the potential risks of *M. equidens* colonizing the Amazon Region are needed. So far, no one knows how or when this alien species was introduced in Brazil.

There are only a few studies on *M. equidens*. They have revealed that adults of this species have carnivorous feeding habits (Krishna-Murthy and Rajagopal

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1990); that adults and larvae can tolerate a wide variations in salinity from freshwater to oceanic levels (Denne 1968, Ngoc-ho 1976), and that *M. equidens* adapts well to degraded environments (Short 2004).

In order to become established in a new environment, the new conditions need to be compatible with the reproduction and developmental requirements of the exotic species. In this context, reproduction is the most critical step, and in the case of *M. equidens*, larvae are dependent on brackish water to complete their development (Ngoc-ho 1976). In this phase, food is one of the most critical items to the success of the larvae (Anger 2001). Therefore, we need to know the proper foods and the proper density of food for the development of *M. equidens*, and how these factors correlate with variations in the anatomical structures of the mouthparts and stomach of the larvae. Furthermore, these data can provide information about the success of reproduction and development of this species in the region.

The ontogeny of decapod larvae is marked by usual changes in dietary habits (Anger 2001). Chu and Shing (1986) found that larvae of *Metapenaeus ensis* are omnivorous in the early larval stages, but later, in the final stages, they become carnivores. Changes in dietary habits among decapod crustacean larvae happen at different stages of development (Anger 2001). In addition, enzyme production in decapod crustaceans determines these dietary changes (Jones et al. 1997). Another factor important to the eating habits of decapod crustaceans is particle size, which can influence food capture and reduce or impair feeding (Anger 2001).

The density of food is also a factor that may determine larval success. Larvae of most decapod crustacean species progressively increase their live food intake as they progress through development (Anger 2001). However, a surplus of food can lead to the deterioration of the conditions where the larvae live, due to an increase in nitrogen compounds (Abrunhosa and Kittaka 1997). By contrast, food limitations can compromise nutritionally depleted larvae, reducing the survival rate and increasing cannibalism (Jones et al. 1997). The relationship between increased larval consumption of *Artemia* in response to an increase in the density of the latter had been previously observed for larvae *of M. rosenbergii* (Barros and Valenti 2003), king crab - *Paralithodes camtschaticus* (Epelbaum and Kovatcheva 2005) and *M. amazonicum* (Maciel et al. 2012). Thus, the success of feeding during larval development depends on the efficient combination of food capture by the larvae and the nutritional quality of the food (Anger 2001). In general, the optimal density of food varies from one species to another and in the same species during different phases of the ontogeny.

Larvae of *M. equidens* have not been subjected to feeding tests to determine their feeding habits. Moreover, the ideal density of food that meets the nutritional requirements of larvae and the morphophysiological aspects of their digestive tract are not known. The present study aims to test different foods and identify their density during the larval development of *M. equidens*.

MATERIALS AND METHODS

Ovigerous females of *M. equidens* were captured with baited traps in the Caeté River, city of Bragança, northeastern Pará, in the Amazon Region (00 58° 58'21" W and 46 ° 44'37" S). After being captured, the animals were transported in constantly aerated water from the place of collection to the laboratory. The animals were then disinfected with formaldehyde 30 mg/L for 30 minutes. Ovigerous females were stored in individual, six liter tanks with brackish water at 16, with constant aeration until the larvae hatched. The females were fed inert diet according to Mallasen and Valenti (1998). Daily, the leftover food and waste were removed by siphoning, with exchange of 80% of the volume of water to avoid the concentration of nitrogen compounds.

After hatching, the larvae were counted and packaged in containers for rearing. The parameters adopted for larval rearing are described below (Table I).

TABLE I
Parameters adopted for larval rearing.

Parameters	Values
Temperature	29.0 ± 1.0°C
Salinity	16.0 ± 0.5
pH	8.0 ± 0.3
Ammonia (NH_4^+), (NH_3)	< 0.1 mg/L
Nitrite (NO_2)	< 0.1 mg/L
Photoperiod	12:12h (light:dark cycle)

Experiment 1 – Feeding Habits

The feeding trials were carried out in 200 mL, aerated containers with 10 larvae/container. The water and the food were changed daily (80%). There were eight treatments (type diet), with ten replicates (total of 80 containers of culture). Treatments (diet offered): (I) starvation, (AL) microalgae, (RO) rotifers, (AN) *Artemia,* (RO + AN) rotifers + *Artemia,* (AL + RO) microalgae + rotifers, (AL + AN) microalgae + *Artemia*, (AL + RO + AN) microalgae + rotifers + *Artemia*. At the end of the experiments, survival and the rate of metamorphosis in larvae and post-larval stage index were evaluated.

Strains of *Chaetoceros gracilis* (Bacillariophyceae) microalgae were reared by using agar gel (fertilized with *Guillard* medium). After the colonies were formed in the Petri dishes, the best set of colonies was selected for the experiment. The exponential growth of algae was conducted using the *Guillard* until obtaining the volume of 2,000 mL.

Rotifers were collected from the Lagoa Salina (46° 50' W, 01° 07' S). In the laboratory, an aliquot of sample was transferred to a Petri dish and rotifers were isolated with the aid of a syringe and a stereomicroscope (objective 40x). Selected organisms were acclimated to growth conditions during 24 hours until reaching the salinity culture (16). The following parameters were monitored: pH 8.0 (± 0.3), salinity 16 (± 1.0), temperature 25°C (± 2.0); microalgae (*Chaetoceros gracilis*) in the density of 4x 10^4 cel/mL and constant aeration. Daily, the leftover food and waste were removed by siphoning.

The density of rotifers was determined by sampling 1 mL of the culture, which was cooled or diluted in fresh water, to slow down the movement of the organisms. After this procedure, rotifers were counted with the aid of a stereomicroscope (40x objective). For the food preference test, we adopted a density of 40 rotifers/mL.

Artemia nauplii were also used in the feeding test. To determine the density of *Artemia* nauplii, a 1 mL sample was taken, diluted with 100 mL of fresh water. From that, 10 sub-samples of 4 mL were removed with a pipette with the tip cut off. All subjects in each sub-sample were counted and an arithmetic average was calculated. This number was multiplied by the volume of fresh water (100 mL). The resulting number was the density of *Artemia* present in 1 mL. To test for food preference, a density of 12 AN / mL was adopted.

Experiment 2 – Density of the Food Selected by Larvae of *M. equidens*

The type of food selected was defined in experiment 1, and the density was determined after observing the amount of the *Artemia* nauplii. We adopted three treatments with five replicates (total of 15 rearing tanks), corresponding to the following food densities: 4 AN / mL, 8 AN / mL and 16 AN / mL. This experiment was conducted in 2-liter tanks furnished with biological filters in a closed dynamic system, with a density of 50 larvae/L. At the end, survival, the percentage of post-larvae, and the dry weight of the post-larvae were evaluated.

The consumption of *Artemia* nauplii throughout the day was assessed at 8:00 am, 12:00 pm and 8:00 pm. With the aid of a pipette with the tip cut off, we withdrew, from each tank, five 4 mL samples. The *Artemia* nauplii present in each sample were counted by visual observation, and the amount of nauplii per 1 ml was calculated. Subsequently, we calculated the average of the five sub-units, and the result was multiplied by the tank volume. The nocturnal food intake was determined by subtracting the number

of nauplii offered, counted at 8:00 pm, from the number of nauplii remaining the next morning, at 8:00 am, before food offer. The daytime intake was calculated by subtracting the number of nauplii counted at 8:00 am (after offering the food) from the number counted at 8:00 pm Previous to offering the food at 08:00 am and pm, we cleaned the tanks by changing the 100 μm screen to a 500 μm, to collect the remaining cysts and *Artemia* nauplii. During this period, we also cleaned the bottom of the tank by siphoning, to collect leftover food and larval excreta. After these procedures, the 100 μm screens were replaced, the temperature was taken, and the flow of water for biological filters was checked.

We collected all organisms from the tanks 26 days after stocking. The numbers of larvae and post-larvae were determined by counting them. From these data and the values of each storage tank, we determined the survival rate, the mortality rate, and the survival curve, as follows:

$$S = N_T / N_0 \quad (1)$$

$$m = - lnS/T \quad (2)$$

$$Nt = N_0 \, . \, e\text{-}mt \quad (3)$$

in which:

S = final survival rate, expressed as a proportion, N_T = number of larvae and PL harvest; N_0 = number of larvae stocked in the tank, m = coefficient of mortality, $-ln$ = natural logarithm, T = days of rearing; Nt = total number of larvae estimated in the tank for each day; e = base of natural logarithm; t = days of culture.

The survival curve was adapted from Krebs (1999) and Winemiller and Dailey (2002) for a single cohort. The equation assumes the mortality rate as constant over time, and has been widely used in biological studies to forecast fishing inventories. As no phases of higher mortality were observed in the hatchery tanks, this curve is acceptable to estimate the amount of animals in the tank every day.

The number of animals contained in the tanks every day was estimated by substituting the desired day in the equation (3). Next, we divided the number of nauplii consumed by the number of animals in the tank on that day to estimate the consumption of *Artemia* nauplii/larva.

Survival, Dry Weight of Post-Larvae

Each experiment (Experiments 1 and 2) was completed when the treatment of at least one experimental group reached 80% of metamorphosis (or higher) to postlarvae. To obtain the dry weight of the postlarvae, we placed the pre-weighed larvae in aluminum paper containers. They were placed in Petri dishes and kept in an oven at 60°C (± 5.0) for 48 hours. Then, the cartridges were transferred to a desiccator and, after two hours, weighed in with an accuracy of 10 μg.

Larval Condition Index (LCI) and Larval Stage Index (LSI) of *M. equidens* larvae

Every 48 hours we verified the Larval Condition index (*LCI*) (adapted from Tayamen and Brown 1999, for *M. rosenbergii* to *M. equidens*). The following items were analyzed: bowel condition, conditions of the hepatopancreas, state of the chromatophores, body coloration, state of the rostrum and setae, the proportion of muscle relative to the intestine, abdominal muscle appearance, melanization and the presence of infesting organisms. Each item checked received a value from 0 to 2, where: 0 = poor, 1 = good, 2 = excellent. The larval condition index (*LCI*) was determined according to the formula below:

$$LCI = \Sigma P / n$$

in which:

P = total number of points assigned to each larvae examined, n = number of larvae analyzed; LCI ranges varied from 0 to 2.

The Larval Stage Index (***LSI***) was adapted from Manzi et al. (1977). For this, the ***LSI***, the post-larvae were not considered. The *LSI* was determined according to the method of weighted average, according to the following formula:

$$LSI = (\Sigma\ ni\ Ei) / n$$

in which:

ni = number of larvae at stage ***Ei***, ***n*** = number of larvae analyzed; ***E*** = larval stage; ***LSI*** = ranges varied from 1 to 10.

STATISTICAL ANALYSES

We used a randomized design for the experiments on eating habits and the density of food offered. In the statistical analysis of the results, we performed tests of normality (Shapiro-Wilk) and homoscedasticity (Brown-Forsythe). As we did not observe a significant deviation from normality and homoscedasticity, we applied the ANOVA *F* test. For the analysis of the intake of *Artemia* we used three treatments (4 AN / mL, 8 AN /mL and 16 AN /mL), and the ANOVA was performed on sub-divided portions. The consumption of nauplii was analyzed using a split-plot ANOVA, with the feeding protocol as the principal factor and larval age and feeding time as sub-categories. We analyzed survival, productivity and dry weight of the postlarvae.

The survival data were transformed by arcsine $\sqrt{x}$, but they were presented as a percentage to facilitate understanding. It was considered that the means differ significantly at $P < 0.05$. Data were analyzed using the SAS software package (version 8.0; SAS Institute).

RESULTS

Food Management Test

The complete larval development happened only in treatments where *Artemia* was offered as a food item. Under the other experiments: starvation (I), and microalgae (AL), all *M. equidens* larvae had died by the day 11. The experiments in which rotifers were offered as food (RO) and rotifers + algae (AL + RO) ended around the day 13, after all larvae were dead. The treatment with *Artemia* (AL + AN, AN and LA + RO + AN) had high survival in which they were differed only to treatment *Artemia* + rotifers (RO + AN) ($P < 0.001$). Statistical differences were observed with respect to other treatments (Table II).

TABLE II

Metamorphosis of post-larvae, survival, and weight of post-larvae subjected to the treatments: *Artemia* (AN) nauplii, *Artemia* complemented with microalgae (AL+AN), *Artemia* complemented with microalgae and rotifers (AL+RO+AN), *Artemia* complemented with rotifers (RO+AN). n= 100 larvae per treatment ($P < 0.05$). Under the other experiments: starvation (I), and microalgae (AL), rotifers (RO) and rotifers + algae (AL + RO) all *M. equidens* larvae had died.

	TREATAMENTS			
	AN	**AL+AN**	**AL+RO+AN**	**RO+AN**
Postlarvae (%)	59 ± 1.72^{bc}	82 ± 1.03^{a}	60 ± 1.05^{b}	44 ± 1.34^{c}
Survival (%)	80 ± 1.33^{a}	93 ± 0.82^{a}	84 ± 1.42^{a}	69 ± 2.46^{b}
Fresh weight (mg)	10.5 ± 0.2^{ab}	13.1 ± 0.11^{a}	$12,2 \pm 0.14^{a}$	9.2 ± 0.18^{b}
Dry weight (mg)	0.95 ± 0.31^{a}	1.09 ± 0.19^{a}	0.95 ± 0.13^{a}	0.63 ± 0.23^{b}

The Larval Condition – *LCI* (close to 2) and the Larval Stage – *LSI* indexes did not differ significantly during the experiment in the treatments containing *Artemia* (Figure 1). The first post-larvae were observed near the 22nd day. The food management test was concluded on the 27th day.

In all treatments of the food management test (Experiments 1) the dissolved oxygen values were close to the saturation point. Ammonia and nitrite, in all observations, were below 0.3 mg / L. The hydrogen potential ranged from 8.0 ± 0.3.

Food Density Test

Intake of *Artemia* nauplii

The *Artemia* intake by the larvae was higher in the 16 AN treatments. The results of the different

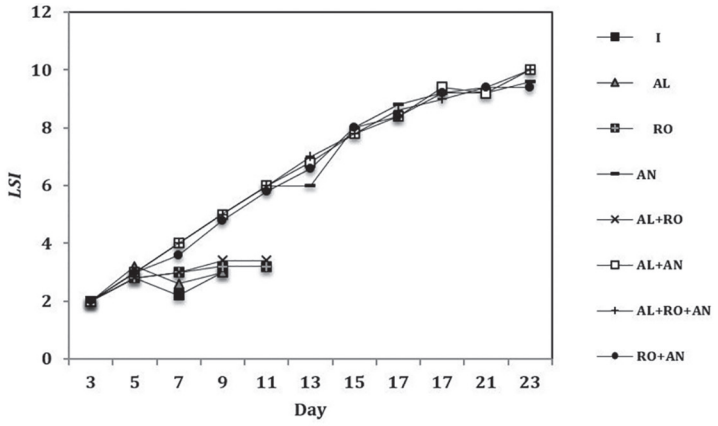

Figure 1 - Larval Stage (*LSI*) indexes subjected to the treatments: *Artemia* (AN) nauplii, *Artemia* complemented with microalgae (AL+AN), *Artemia* complemented with microalgae and rotifers (AL+RO+AN), *Artemia* complemented with rotifers (RO+AN). The complete larval development happened only in treatments where *Artemia* was offered as a food item, and the *LSI* did not differ significantly during the experiment. Under the other experiments: starvation (I), and microalgae (AL), all *M. equidens* larvae had died by the 11th day. The experiments in which rotifers were offered as food (RO) and rotifers + algae (AL + RO) ended around day 13th, after all larvae were dead. n = 50 larvae per treatment.

treatments differed statistically, throughout the days of the experiment, and between daytime and night-time (Table III).

TABLE III

The consumption of *Artemia* nauplii was analyzed using a split-plot ANOVA, with the feeding protocol as the principal factor and larval age and feeding time as sub-categories. We considered the differences statistically significant when $P < 0.05$.

Factors and interactions	F	*P*
Treatment	1703.52	0.0001**
Replicate (Treatment)	3.01	0.0005**
Day	102.23	0.0001**
Day x Treatment	10.11	0.0001**
Replicate (Day x TR)	0.67	0.9997
Hour	17853.2	0.0001**
Hour x Treatment	1050.49	0.0001**
Day x hour	109.30	0.0001**
Day x Hour x Treatment	11.42	0.0001**

The larvae of *M. equidens* began to eat at development stage II, which they generally reach on the second day of life. In this phase, they consumed more *Artemia* nauplii during the daytime and at the concentration of 16 AN / mL (Figure 2).

Larvae of *M. equidens* ate *Artemia* nauplii mostly during the day, and increased their intake during development and in response to increasing density of food supply (Figure 2). On the fifth day of culture, when most larvae were in Stage IV, the daily intake of *Artemia* decreased. At night there was no increase in the intake of *Artemia*, independent of the density offered, except between days 6 and 7 of culture. During this period, most larvae were between stages IV and V.

The difference in the intake of *Artemia* was significant between treatments (4 AN /mL, and 8 AN /mL and 16 AN /mL) and between daytime and night-time (Table III). In the later stages of development, a decrease in food intake in the different treatments was not observed, regardless of the density offered.

Larval Condition Index (LCI) and Larval Stage Index (LSI)

With respect to the Larval Condition Index *(LCI),* no significant differences in the results of treatments 8 AN/mL and 16 AN/mL were found (close to 2). However, the results of treatment 4 AN / mL differed significantly from those of other treatments from day 14 (1.6 ± 0.2). There were no statistical differences in the Larval Stage Index (*LSI*) within

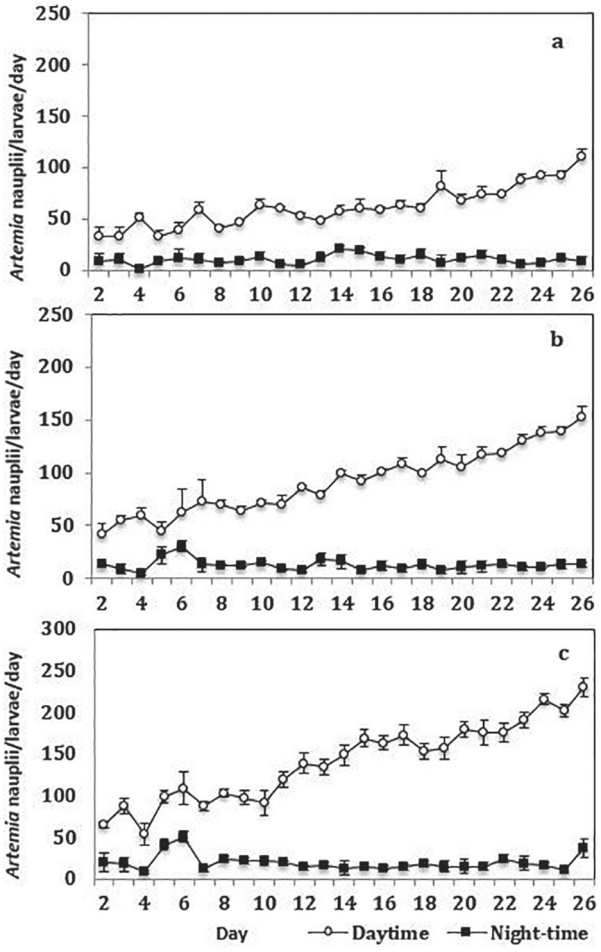

Figure 2 - The consumption of *Artemia* nauplii, mean daytime and night-time ingestion by *Macrobrachium equidens* larvae during the study period in the following densities: **a**) 4 AN / mL, **b**) 8 AN/ mL and **c**)16 AN / mL.

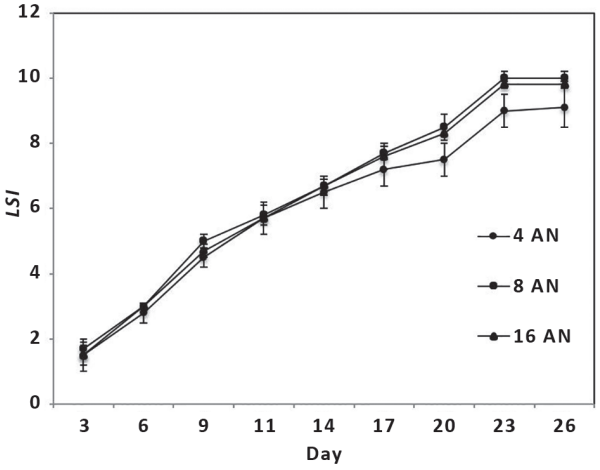

Figure 3 - Larval Stage (*LSI*) indexes subjected to the treatments with food densities (*Artemia* nauplii - AN): 4 AN / mL, 8 AN / mL and 16 AN / mL. There were no statistical differences *LSI* within 14 days, thereafter, the 4 AN/ml treatment was statistically different (P = 0.0049) from the other treatments (8 and 16 AN / mL).

14 days, stage VI (Figure 3). Nevertheless, after this period delay occurred in the LSI for larvae of the treatment 4 AN/mL (P = 0.0049) only.

There were no significant differences in survival rates, metamorphosis to postlarvae, productivity and weight between the 8 AN and 16 AN treatments (Table III). However, the results of the 4 AN / mL treatment had lower values and were statistically different from those of other treatments (P <0.0001) (Table IV).

TABLE IV

Survival, postlarvae, productivity and weight of subjected to the treatments, food densities: 4 AN /mL, 8 AN / mL and 16 AN / mL. Different letters on the same line indicate significantly different means (Tukey test, P< 0.05).

	4 AN	8 AN	16 AN
Survival (%)	56.8 ± 5.09[b]	78.4 ± 6.51[a]	73.8 ± 3.69[a]
Postlarvae (%)	62.4 ± 3.57[b]	83.6 ± 2.96[a]	74.8 ± 2.28[a]
Productivity (PL/L)	20.1 ± 2.86[b]	35.3 ± 5.17[a]	31.4 ± 3.03[a]
Fresh weight (mg)	6.58 ± 1.10[b]	9.97 ± 1.20[a]	10.38 ± 0.64[a]
Dry weight (mg)	0.39 ± 0.13[b]	0.93 ± 0.16[a]	0.98 ± 0.71[a]

Larval Rearing

Larval rearing took place in a rectangular, 2 liters tank with brackish water (16) in a closed system dynamic, and a larval density of 50/liter (Experiment 2). We controlled the physic and chemical parameters of the water: pH (8.0 ± 0.3), temperature (29 ± 1.0°C), salinity (16 ± 0.5 mg/L), dissolved oxygen (6.5 ± 0.5 mg/L), ammonia (0.1

± 0.025 mg/L) and nitrite (0.1 ± 0.025 mg/L). The complete development of *M. equidens* consisted of 10 Zoea stages and one juvenile stage. Survival was 84%. The first postlarvae were observed near day 23 of the experiment and larval development lasted 23-26 days, until the larvae reached the juvenile stage.

DISCUSSION

Successfully rearing a crustacean species depends on the quality of the water used and the availability of appropriate food. To date, no experiments on the feeding of *M. equidens* larvae had been performed, which makes this study the first to ascertain the food habits of this species, and how it relates to food density.

Given that the water quality in all experiments was deemed adequate, we can attribute the different results we observed to the management of food. Factors that may have interfered in the process were the size and speed of the prey, as well as the ease with which the larva captures, assimilates and digests it. Moreover, larvae undergo changes throughout their ontogenetic development, which impacts their ability to find foods that meet all the requirements of each larval stage (Liu et al. 2007).

M. equidens larvae have carnivorous habits. Furthermore, they have certain food requirements. This idea was corroborated in our experiment by the fact that only larvae, which were given *Artemia* nauplii, completed their full development to post-larvae. Possibly, *M. equidens* larvae are able to capture and digest *Artemia* during all phases of their development, even though, at some point, the relationship between the size of the prey and that of the predator might be disadvantageous to the latter. Barros and Valenti (2003) observed that larvae of *M. rosenbergii*, in their early stages, consume only the abdomen of *Artemia*, suggesting that the prey is too large for the predator. Nevertheless, *M. rosenbergii* larvae can be reared successfully on *Artemia* as their only food source (Lavens et al. 2000). According to Anger (2001), when a predator is able to feed on large prey items, the energy gain is higher, which may be beneficial to decapod larvae.

Feeding rotifers to *M. equidens* larvae (*Brachionus plicatillis*) was not appropriate from the early zoae stages. This may have been due to the fact that *M. equidens* cannot efficiently capture or digest and assimilate this food source. The main evidence for this is a reduction in the larval condition index from the fourth day of culture, which appeared similar to the condition of larvae kept unfed or larvae that were fed microalgae. Larvae on a rotifer diet only reached the third larval stage, when they probably were still using yolk reserves. Furthermore, individuals fed rotifers + *Artemia* also had reduced survival rate and weight, thus suggesting that the simple presence of rotifers in the tank had some kind of detrimental effect on *M. equidens*. A similar situation was observed for larvae of *Scylla serrata*, which was attributed to a possible visual confusion of the larvae, resulting in the reduction in prey capture (Ruscoe et al. 2004). In addition, rotifers do not meet the nutritional needs of fish larvae (Atlantic cod), and their presence increases the risk of bacterial contamination, reducing water quality (Hamre et al. 2008). In this study, *M. equidens* was reared at low densities, the density of food was controlled, and water was exchanged daily, probably minimizing the effects of water quality. Therefore, it is possible that larvae of *M. equidens* in the experiments that included rotifers were nutritionally compromised.

Our results revealed that *Chaetoceros gracilis* microalgae are not a food item in the diet of *M. equidens*; however, their occurrence with *Artemia* promoted larval survival and improved the quality of the yield of individuals fed rotifers and *Artemia*. The benefit provided by the algae, however, is unknown. Some studies have shown that they exert an indirect effect, stimulating the release of digestive enzymes (fish larvae of *Sciaenops ocellatus*), substances that stimulate ingestion of food (betaine and free amino acids), and a possible direct effect, by providing

micronutrients (vitamins) (Lazo et al. 2000). Felix and Surdharsan (2004) reported that the addition of glycine betaine as attractants for *M. rosenbergii* juveniles improves the final weight and survival rates of the latter. Harpaz (1997) reported that the presence of betaine in a tank with juveniles results in 17% growth. In addition, compared to other microalgae, *C. gracilis* stands out due to its high content of chlorophyll *a*, proteins, soluble carbohydrates, and total free amino acids (Moura-Júnior et al. 2006). Another possible explanation is that these algae not only nurture the *Artemia* nauplii, but that they also contain nutrients that get transferred to the *M. equidens* larvae through the food chain.

We observed that the *M. equidens* larvae increase their intake of *Artemia* nauplii in response to increasing density of supply, as well as during ontogeny. Similar results were observed for larvae of different species of crustaceans: *Paleamon serratus* (Yúfera and Rodríguez 1985a), *Palaemonetes varians* (Yúfera and Rodríguez 1985b), *Metapenaeus ensis* (Chu and Shing 1986), *Scylla serrata* (Zeng and Li 1992), *Ranina ranina* (Minagawa and Murano 1993), *Panopeus herbstii* (Welch and Epifanio 1995), *Zysmata wurdemani* (Zhang et al. 1998), *S. serrata* (Suprayudi et al. 2002), *M. rosenbergii* (Barros and Valenti 2003) and *M. amazonicum* (Maciel et al. 2012). In most of these studies, higher rates of survival were associated with increased ingestion rates and a reduction in the duration of the intermolt. For *M. equidens*, we found that the low density of food could be limiting for larval development, decreasing survival rate and weight. Moreover, higher density (16 nauplii/mL) of food was correlated with increased food intake, but not with longer survival rates, LCI, LSI and weight of the post-larvae with respect to an intermediate density (8 nauplii/mL). A similar situation has been previously observed in larvae of *Lysmata wurdemanni*, wherein the increased intake did not lead to increased survival and may even have had a negative impact (Zhang et al 1998). It is possible that the larvae of *M. equidens* have an opportunistic behavior in response to density of supply, and do not become satiated. The absence of satiety had also been previously observed in larvae of *Palaemon serratus* (Yúfera and Rodríguez 1985a) and *Palaemonetes varians* (Yúfera and Rodríguez 1985b), corroborating our hypothesis.

When we analyzed food intake throughout the day, we found that *M. equidens* larvae fed preferably during daytime, and that night-time intake was negligible. This behavior had been previously reported for larvae of another crab species, *Ranina ranina* (Minagawa and Murano 1993), confirming our results. It is possible that vision has an important role in prey capture. However, this hypothesis has been questioned by several authors, who have regarded capture success as depending on a chance encounter between predator and prey (Moller 1978, Anger 2001, Barros and Valenti 2003). Recently, it was found that larvae of *M. rosenbergii* have opposed eyes, which are adapted to an illuminated environment, providing greater visual acuity than the eyes of the adults of the species (Cavalari 2006). Moreover, positive phototropism has been reported for decapod larvae (Lin 1997, Anger 2001), which swim about more intensely during daylight, increasing the likelihood of finding prey. The hypothesis we advance for *M. equidens* larvae predicts that vision is critical for prey capture, because in the absence of light (night), food intake fell dramatically, even in the presence of high densities of food, which increase the chance of a predator and prey encounters. Another hypothesis predicts that there is an endogenous trophic rhythm associated with the feeding behavior and the cycle of molt. Future studies should be conducted to test these two hypotheses.

During the ontogenetic development, larvae undergo anatomical and physiological changes that might interfere with their ability to capture prey. In larvae of *M. equidens*, we observed that the pleopods and statocysts arose from stages VI

and VII, respectively, increasing the larval ability to shift and to perceive the environment. Zhang et al. (1998) reported that when larvae of *Lysmata wurdemanni* reach stages IV to VII, their feeding rate is less influenced by an increase in food density than by their increased capacity to move and capture prey. Further, the acceptance and ingestion of a diet may be influenced by the maturity of the digestive tract and by the physical and chemical properties of the food itself, as well as by the larval ability to perceive signals (Jones et al. 1997).

The present study strongly suggests that larvae of *M. equidens* are essentially carnivores because they reach the juvenile stage when fed *Artemia* nauplii only. We also verified that the larvae feed mainly during the daytime, and they are opportunist with respect to the density of food offered. Such behavior may attribute to invasive species an important role as a competitor or predator of native species that utilize the estuarine area to complete their reproductive cycle (Maciel et al. 2011). Beyond this, the trophic diurnal rhythm suggested for *M. equidens* is in accordance with a more abundant prawn in the Amazon region, *M. amazonicum*, (Maciel et al. 2012). Recent studies have demonstrated an expansion of the *M. rosenbergii* in the Amazon coast and a real risk of colonization in that region was presumed (Iketani et al. 2011, Silva-Oliveira et al. 2011). For *M. equidens*, the status of invasion in the Amazonian area is still unknown therefore further studies are necessary in order to clarify this issue.

RESUMO

No presente estudo foram realizados experimentos sobre alimentação, com a espécie de camarão de água doce *Macrobrachium equidens*. Foram, testados o tipo de alimento e a densidade de alimento adequada para o desenvolvimento das larvas. Para o experimento sobre o tipo de alimento, 8 tratamentos foram realizados: (I) inanição, (AL) microalgas, (RO) rotíferos, (NA) náuplios de *Artemia,* (RO + NA) rotíferos + *Artemia*, (AL + RO) microalgas + rotíferos, (AL + NA) microalgas + *Artemia,* (AL + RO + NA) microalgas + rotíferos + *Artemia*. Para o experimento sobre a densidade de alimento, foi utilizado o tipo de alimento que resultou em uma elevada taxa de sobrevivência no experimento anterior. Três tratamentos foram realizados: 4, 8 e 16 náuplios de *Artemia*/mL. A taxa de alimentação durante o desenvolvimento das larvas foi observada. A sobrevivência, o peso e o percentual de juvenis de cada experimento de alimentação foram determinados. Foi identificado que as larvas de *M. equidens* são carnívoras, no entanto, houve uma seletividade do tipo de alimento, pois as larvas concluíram o seu ciclo de zoea para a fase de juvenil somente quando os náuplios de *Artemia* foram disponibilizados. Verificou-se também que as larvas se alimentam preferencialmente durante o período diurno, e são oportunistas em relação à densidade do alimento ofertado.

Palavras-chave: Palaemonidae, dieta, preferência alimentar, alimento vivo.

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