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Feeding habit of the Amazon river prawn *Macrobrachium amazonicum* larvae

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Abstract

To investigate the feeding habit of *Macrobrachium amazonicum*, three experiments were carried out assessing the stage at which larvae start exogenous feeding, the acceptance of inert food by different larval stages and the ratio between live and inert diet ingested by larvae at each larval stage. In the first experiment, newly hatched larvae were kept in 500-mL beakers and fed from stages I, II or III onward. Larval survival was not affected by the larval stage at which exogenous feeding started, but mean weight gain was lower when food was offered from stage III onward. In the second experiment, 60 larvae from each stage (I to IX) were fasted for 2 h and then fed on inert diet in excess. Only larvae from stage IV onward accepted this inert diet. In the last experiment, newly hatched larvae were stocked in a larviculture tank and fed daily on both *Artemia* nauplii and inert diet. After 15 min, food content in the digestive tract of individual larvae was analyzed under stereomicroscopy. Larvae in stage I did not feed, while live food was accepted from stage II onward and inert food from stage III onward. Larvae in stages IV, V and VI accepted both foods with no preference, while inert food was predominant in stages VII to IX. In conclusion, to feed *M. amazonicum* larvae on *Artemia* before stage II or on inert diet before stage IV is unnecessary. It increases production costs and may impair water quality. From stages IV to VI, feeding on *Artemia* and inert diet is probably necessary, while inert diet should be the main food item from stage VII onward. This schedule may optimize feeding management and production costs.

Keywords: Freshwater prawn; *Macrobrachium amazonicum*; Larviculture; Feeding

1. Introduction

In Brazil, the major cultured freshwater prawn is the exotic *Macrobrachium rosenbergii* (Valenti and Moraes-Riodades, 2004). Although there is no evidence of any negative ecological impact of this species, there are benefits to use local species rather than exotic ones both for genetic and environmental reasons. It increases the sustainability and local acceptance of the freshwater prawn farming activity. Therefore, studies on native species are needed to assess their commercial viability and develop relevant farming technology.

Macrobrachium amazonicum is the South American prawn with the greatest potential for aquaculture (Kutty et al., 2000; New, 2005). This prawn is widely distributed in estuaries and mainly interior areas, from Venezuela to Argentina. In north and northeast Brazil, *M. amazonicum* is very important to artisan fisheries (New et al., 2000). Its meat is firmer and the flavor more pronounced than *M. rosenbergii* (Valenti et al., 2003).

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Since 2001 a multidisciplinary and multi-institution program has developed technology to culture *M. amazonicum* in Brazil. Preliminary results have showed that larval phase lasts about 20 days, survival rate is high and this species presents rapid growth and development in ponds as well as high resistance to diseases.

Only a few studies have been carried out on *M. amazonicum* larvae (Guest, 1979; Mcnamara et al., 1983; Gamba, 1984; Moreira et al., 1986; Zanders and Rodríguez, 1992; Moreira and Odinetz-Collart, 1993), and despite its high potential for aquaculture, the conditions for its larviculture are still not established. Some experimental trials have been performed based on technology used for *M. rosenbergii*. Although they are species of the same genus, they originated from distant geographical sites, suggesting that different biological features may have evolved leading to different biological requirements for *M. amazonicum* aquaculture. Thus, management procedures for *M. amazonicum* need more detailed investigation.

One of the main factors affecting larviculture of freshwater prawns is feeding strategies, which must be adjusted to the behavior and nutritional needs of the larvae (Loya-Javellana, 1989). However, information on the digestive capacity, digestive processes and nutritional requirements of freshwater prawn larvae is still scarce. Besides this, larvae pass through several developmental stages, each one presenting specific morphophysiological, behavioral and probably nutritional characteristics (Sorgeloos and Léger, 1992; Lavens et al., 2000). Thus, each larval stage must be considered before setting up a feeding schedule for larviculture of any prawn (Loya-Javellana, 1989). For *M. amazonicum*, no studies on the acceptance of different diets during the larval phase have been reported.

The use of *Artemia* nauplii for feeding prawn larvae has advantages, such as easy management, adequate size and high-content of essential nutrients available for predator (Lavens et al., 2000). However, some authors have argued that *Artemia* nauplii do not provide all the nutrients needed in the final larval stages of *M. rosenbergii* (Daniels et al., 1992; Barros and Valenti, 2003a) and some species may not be strictly carnivorous during all larval phases. In addition, the cost of *Artemia* cysts has increased and, as a consequence, the post-larvae production cost increases as well. Thus, feeding managements using alternative diets to replace or complement *Artemia* nauplii feeding should be more suitable and may decrease production costs. The search for these procedures demands detailed studies on larvae feeding habits, indicating exactly the larval stage at which the different diets should be provided (Barros and Valenti, 2003a).

Considering the above rationale, the present study evaluated the feeding behavior of *M. amazonicum* larvae in response to live and inert feeds throughout larval development. A first experiment investigated the stage at which larvae need exogenous feeding; a second one evaluated the acceptance of inert food by the different larval stages; and a final experiment assessed the ratio between live and inert food ingested by larvae at each larval stage.

2. Materials and methods

The study was conducted in the Crustacean Sector of the Aquaculture Center (CAUNESP) at the São Paulo State University, Jaboticabal, Brazil. *M. amazonicum* larvae were obtained from ovigerous females reared in earthen ponds. These animals were produced in the prawn hatchery of CAUNESP from broodstock originating from northeastern Pará state, Brazil (1° 13′25″S, 48° 17′40″W) in 2001. Larval stages were identified according to Guest (1979).

2.1. Experiment 1 — starting exogenous feeding

This experiment was set up according to a completely randomized design with three treatments (larval stages at which feed starts to be offered) and three replicates. Newly hatched larvae of *M. amazonicum* were reared in 9 beakers containing 500 mL brackish water each (10 salinity; 50 larvae/beaker) for 5 days. *Artemia* (20 nauplii/mL) was given to larvae from stages I (treatment 1), II (treatment 2) or III (treatment 3) onward once in the afternoon. The beakers were supplied with constant aeration and maintained on a black tray with warm water (30.2±0.5 °C). The water in the beakers was completely changed every day to assure suitable water quality. After larvae reached the zoea IV stage (5 days after stocking), culture was finalized; all larvae were counted and weighed. Survival was calculated by the ratio between the final and initial number of larvae in each beaker. To determine dry weight, larvae were rinsed with distilled water, dried on filter paper, transferred to pre-weighed cartridges (10 larvae/cartridge) and dried in an oven (60 °C) for 24 h and after that maintained in a desiccator for 2 h. The dried larvae were then weighed on an analytical balance (to 1 μg).

2.2. Experiment 2 — acceptance of inert food

This experiment was set up to determine the frequency of inert food ingestion in each of the nine larval stages. Experiment was designed as model II for a

two-way frequency table (Sokal and Rohlf, 1995). Larval stage was one criterion and the ingestion of food was the second one. Therefore, a 9×2 contingency table was obtained. Twelve replicates were performed for each larval stage.

The inert diet consisted of moist feed (Valenti and Daniels, 2000) and was composed of chicken egg (34.0%), mussels (10.0%), marine fish (10.0%), dried milk (4.0%), wheat flour (2.0%), cod liver oil (0.8%), vitamin and mineral supplement (1.4%) and water (37.8%). These ingredients were weighed, mixed in a blender and cooked in a water bath to pudding consistency. After cooling, the mass was cut into small pellets and frozen at −18 °C. Before the pellets were given to the larvae, they were weighed and sieved (stainless steel mesh; 425 μm). As determined by Mallasen and Valenti (1998), the nutritional value of this diet is approximately 45% crude protein, 24% ether extract, 23% nitrogen-free extract, 9% minerals, 18% original dry matter and 5000 kcal/kg gross energy.

A larviculture was established to supply larvae at different stages. A black rectangular tank (50 L brackish water, 10 salinity) was stocked with 4000 newly hatched larvae (80 larvae/L). It was supplied with constant aeration, biological filter, heater and thermostat. Light:dark cycle was 12:12 h (light from 6:00 to 18:00). Water quality was maintained within the accepted range indicated for *M. rosenbergii* by Correia et al. (2000). Temperature was measured twice a day (morning/afternoon) and controlled to about 31 °C. Dissolved oxygen, salinity, ammonia and nitrite were monitored twice a week. Larvae fed on *Artemia* nauplii and, from stage V onward, fed also on inert diet (0.6 mg inert diet/larvae and 5 *Artemia* nauplii/mL in the beginning and 1.7 mg inert diet/larvae and 15 nauplii/mL by the end of the experiment). Feces and leftover food were daily siphoned. Water quality parameters were: temperature 31.1±0.7 °C, dissolved oxygen 6.30±0.37 mg/L, salinity 10.0±0.3 ppt, total ammonia nitrogen 49.2±8.6 μg/L and total nitrite nitrogen 51.5±8.2 μg/L. Survival (larvae+post-larvae) was 67% after 20 days.

During larval culture, larvae from each zoeal stage (zoea I to zoea IX) had been selected for the tests. As soon as most of the larvae population into the rearing tank attained a particular stage, sixty larvae at this stage were transferred to 12 beakers containing 80 mL of brackish water (10 salinity; 5 larvae/beaker). Water was supplied by the rearing tank and filtered through a nylon net (125-μm mesh); therefore it is assumed that the water in the beakers presented the same characteristics of the rearing tank. Black opaque partitions prevented larvae visual contact through the beakers. Aeration was sufficiently strong to keep diet suspended in the water column.

Larvae were deprived of food for 2 h and then fed once with inert diet in excess. After 15 min, all larvae were collected, put in a Petri dish and examined under a stereomicroscope to determine the incidence of inert diet inside the digestive tract. Digestive content was classified into a) absence of food (both stomach and intestine were empty) or b) presence of food (stomach and/or intestine were partially or completely full). Data from the 12 beakers were pooled and the percentage of acceptance of inert feed was determined by the ratio between the number of larvae in a particular larval stage with feed presence and the total number of larvae observed (N=60).

2.3. Experiment 3 — ingestion frequency of live and inert diet

This experiment was set up to determine the frequency of food ingestion of both *Artemia* nauplii and inert diet during larval development. Experiment was designed as model II for a two-way frequency table (Sokal and Rohlf, 1995). Larval stage was one criterion and the ingested food was the second one. As ingested food was classified in four categories, a 9×4 contingency table was obtained. For each larval stage, 50 larvae were sampled and analyzed.

Newly hatched larvae were stocked in a rectangular black tank containing 50 L brackish water (10 salinity, 80 larvae/L) in a closed recirculation system, with conditions and management as described for larval rearing tank in experiment 2. Water quality parameters were: temperature 30.5±0.4 °C, dissolved oxygen 6.92±0.23 mg/L, salinity 10.0±0.5 ppt, total ammonia nitrogen 53.8±44.1 μg/L and total nitrite nitrogen 52.0±43.2 μg/L. Survival (larvae+post-larvae) was 71% after 20 days.

Inert diet (the same used in experiment 2) and *Artemia* nauplii, were provided in excess simultaneously, twice each morning (8:00 and 10:00 AM) during all culture. The initial meal was 1.6 mg of inert diet/larvae and 15 nauplii/mL at day 1; it was increased almost daily up to 5.0 mg inert diet and 45 nauplii/mL at day 20. During larval culture, larvae from each zoeal stage (zoea I to zoea IX) had been selected to analysis. As soon as most of the

Table 1
Survival and dry weight (mean±sd) of *M. amazonicum* (zoea IV) fed from stages I, II to III on *Artemia* nauplii

Initial feed	Survival (%)	Dry weight (μg)
Stage I	73.3±7.0a	107.7±5.1a
Stage II	74.7±2.3a	99.6±7.2a
Stage III	68.7±6.1a	78.0±1.8b

Means followed by the same letter in the same column did not differ at 0.05 level (ANOVA).

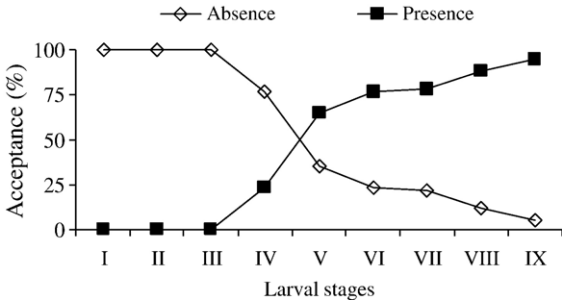

Fig. 1. Acceptance (%) of inert food by the larval stages of *M. amazonicum* ($N=60$). Food content in the digestive tract was classified as either presence or absence of inert food. Association between acceptance of inert diet and larval stage are significantly different at 0.001 level (chi-square test of independence).

larvae population into the rearing tank attained a particular stage, fifty larvae at this stage were randomly sampled 15 min after the first feeding (8:00 AM). These larvae were analyzed under a stereomicroscope for determination of food content in the digestive tract. Food content was classified into a) *Artemia* nauplii; b) inert diet; c) *Artemia*+inert diet; and d) absence of food. For each larval stage, the percentage of food content was estimated from the ratio between the number of larvae with equal food content and the total number of larvae ($N=50$). The size ratio between prey and stage VII *M. amazonicum* larvae was calculated. Body length of *M. amazonicum* larvae is 6.024±0.133 mm (Pérez, 1984) and the newly hatched *Artemia* is about 0.430 mm (Alam et al., 1995).

3. Statistical analyses

Data on weight and survival obtained in experiment 1 for larvae fed from stages I, II to III were compared by ANOVA, followed by Tukey's test. Data are shown as mean values, but survival data were transformed by arcsine square root for statistics. In experiments 2 and 3, the frequencies of larvae at each of any category of ingested food by larval stages were compared using the chi-square test of independence (Sokal and Rohlf, 1995). All the analyses were performed by the software "Statistical Analysis System — SAS" (version 8.02) and the significance level was set at $p<0.05$.

4. Results

In experiment 1, larvae from the three treatments were able to reach zoea IV stage. Survival was not affected by the stage *Artemia* nauplii was provided for larvae feed (Table 1). However, larvae fed from stage III onward had lower mean dry weight than those fed from either stage I or II (Table 1).

In experiment 2, a significant association between acceptance of inert diet and larval stage ($p<0.001$) was observed. Ingestion of inert food started at stage IV, but only 23% of the larvae in this stage had inert food in the digestive tract (Fig. 1). At stage V, the acceptance of inert diet had almost tripled, reaching 65% of the tested larvae; at stage VI it was 75%, and at stage IX 95%.

In experiment 3, food content of the digestive tract changed significantly according to larval stage ($p<0.001$) (Fig. 2). At stage I, the digestive tract was completely empty. *Artemia* was accepted from stage II (20%) onward, and in stage III a low percentage of larvae (14%) fed on inert diet. Larvae in stages IV, V and VI presented similar food ingestion ($p>0.05$): 43% of larvae ingested *Artemia*, 31% ingested inert diet and 20% ingested both. Therefore, 51% of the larvae in stages IV to VI ingested inert diet. From stages VII to IX, the number of larvae accepting exclusively inert food exceeded significantly those feeding exclusively on *Artemia* nauplii. At stage VII, the size ratio between *Artemia* nauplii and *M. amazonicum* larvae was 0.07.

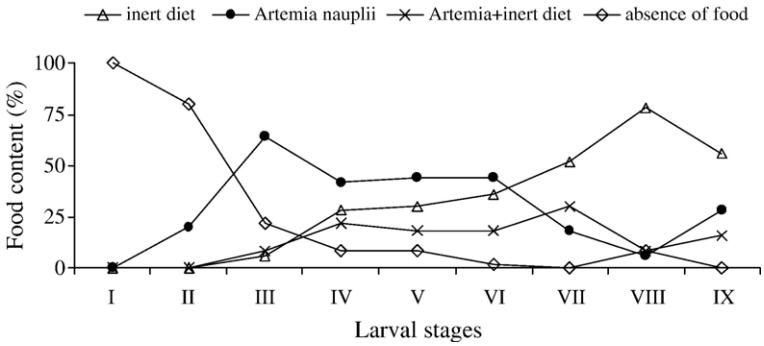

Fig. 2. Frequency of *M. amazonicum* larvae (%) according to food content in the digestive tract in different larval stages ($N=50$). Larvae were grouped into four categories according to the type of food ingested: a) *Artemia* nauplii; b) inert diet; c) *Artemia*+inert diet; and d) absence of food. Type of food ingestion varied significantly in the larval stages at 0.001 level (chi-square test of independence).

5. Discussion

M. amazonicum undergo important changes in feeding habit during larval development, which should be considered for aquaculture purposes. Zoea I are lecithotrophic, while zoea II may be lecithotrophic or zooplanktotrophic, i.e. presents facultative lecithotrophy. Zoea III are zooplanktotrophic (carnivorous), whereas zoea IV, V and VI are clearly omnivorous, feeding zooplankton and particulate organic matter with no preference. Although zoea VII, VIII and IX are also omnivorous, an apparent preference to particulate organic matter was observed. These changes show that the larvae become more generalist throughout the ontogenetic development, and may consume food according to availability. This increase in the feeding spectrum since stage IV (4–5 days after hatching) confers the possibility to explore different feed resources in a natural environment during most larval phase, which is about 20 days. It suggests that *M. amazonicum* larvae may be mainly polytrophic in hatchery tanks and therefore broad potential feed sources may be used. In spite of this, the specific feeding habit in each larval stage should be considered to establish an effective feeding schedule.

Larvae of *M. amazonicum* start exogenous feed from zoea II stage as already described for *M. rosenbergii* (Moller, 1978; Barros and Valenti, 1997). However, only a few zoea II ingest exogenous food, differing from *M. rosenbergii*, whose larvae typically eat at stage II (Barros and Valenti, 2003a). Possibly, internal nutrient stores may be enough to nourish *M. amazonicum* larvae in the first or in the two first stages depending on the amount of yolk stored in the eggs during maternal ovary maturation. Feeding larvae from stage II does not impair *M. amazonicum* development. On the other hand, starting feeding from stage III may impair development of some poor yolk larvae during stage II, decreasing populational mean weight at stage IV, as observed in this work. Data obtained in the present paper suggest that *M. amazonicum* larvae should be fed on *Artemia* nauplii from stage II onward to obtain the best development of all larvae. However, taking into account the high costs of live food, studies should be performed to evaluate if it is more profitable to start feeding from larval stage III onward, taking advantage of the facultative lecithotrophy of zoea II.

Inert diet was accepted from stage IV onward in the beakers and from stage III on in the tank (experiments 2 and 3, respectively). This difference is probably because dispersion and permanence of inert food in the water column is higher in the tanks, which increases the opportunity of larvae to capture food (Barros and Valenti, 2003a). Similarly, the higher dispersion of *Artemia*, which swims actively, may facilitate larvae to encounter this food. Although consumption of inert food started from stage III, 50% of the larvae were feeding on this diet only upon reaching stage IV. Barros and Valenti (2003a) studying *M. rosenbergii* found that a 50% acceptance of inert food was obtained only when larvae reached stage VII. These results indicate that *M. amazonicum* becomes omnivorous earlier than *M. rosenbergii* because, despite having a different number of larval stages, the external morphology indicates that stage IV in *M. amazonicum* is close to stage IV in *M. rosenbergii*.

The higher acceptance of inert diet from stage IV onward may be a consequence of the development of sensorial structures and appendages, which may allow the perception of food in the water column and the active movements to catch it. Throughout the larval phase, *M. amazonicum* larvae develop antennules, endopodites and mouth parts (Guest, 1979; Pérez, 1984), which may present tactile and chemical receptors as what normally occur in crustacean larvae (Ache, 1982). Thus, larvae from stage IV onward may be best able to detect and select food as well as swim towards it, rather than by chance encounter. When larvae reached stages VII and VIII, they ingested inert diet rather than *Artemia*. This may be due to an increase in visual acuity in the last stages (Araujo, 2005), an increase in inert diet attractiveness during omnivorous feeding phase and/or difficulty in predating small prey such as *Artemia* nauplii. It is possible that larvae have difficulty in capturing live food because the size ratio between prey and predator at stage VII was 0.07. According to Barros and Valenti (2003b), the best ratio between prey and predator size is 0.2 for *M. rosenbergii*, and capture becomes more difficult as this value decreases. Further studies investigating this issue are still needed.

The development of the digestive tract and enzyme activity may also account for the consumption of inert diet rather than *Artemia* in stages VII to IX. The first *M. rosenbergii* larval stages are carnivorous and have low concentrations of amylase and trypsin, but at stage VI (which corresponds to stage V of *M. amazonicum*) the production of digestive enzymes increases abruptly (Kamarudin et al., 1994; Kumlu and Jones, 1995). Even for *M. rosenbergii*, this peak coincides with decrease in yolk, and increase in hepatopancreas and diverticula of the proximal portion of the midgut (Jones et al., 1993; Kumlu and Jones, 1995; Lavens et al., 2000). If the development of the digestive tract in *M. amazonicum* is similar to that in *M. rosenbergii*, the low acceptance of

inert diet in the first stages (II and III) and higher consumption of inert diet in stages VII and VIII may be due to the digestive enzymes available during larval development. According to this idea, stages IV to VI are transitory and thus larvae consume both live and inert diets with no preference. Studies investigating the formation of glands and secretory epithelial cells during larval development of *M. amazonicum* could elucidate this hypothesis.

Changes in the mandible structure of *Macrobrachium* larvae may occur simultaneously to the change of feeding behavior from carnivorous to omnivorous (Jones et al., 1997). However, this was not supported by the present study. Pérez (1984) reported that *M. amazonicum* mandible has teeth from stages II to IX, which have been associated to the carnivorous habit. However, a clear change from carnivorous to omnivorous habit throughout the larval development was observed in this study so mandible structure is not related to the feeding habit in *M. amazonicum*.

In conclusion, feeding *M. amazonicum* larvae on *Artemia* before stage II or on inert diet before stage IV is unnecessary. It increases production costs and may impair water quality. From stages IV to VI, feeding on *Artemia* and inert diet is probably indispensable, while inert diet should be the main food item from stage VII onward (11 days after stocking). This management procedure may represent savings in the use of *Artemia* cysts that accounts for more than 50% of the variable costs in freshwater prawns commercial hatcheries (New, 2002). The perspective for total or partial replacement of *Artemia* nauplii from stage VII onward is evident, as larvae present a clear preference for inert diet in the last larval stages. Further studies should be performed to evaluate the nutrient composition of inert diet and find a formulation, which match the *M. amazonicum* requirements.

Finally, it should be noted that the results obtained from beakers and small tanks might not be the same as in commercial hatchery tanks, which are larger and different in shape. In addition, larvae behavior may be affected by water quality and management strategy. Therefore, mass rearing culture should be tested. Results obtained in the present work may subsidize future researches and serve as a guideline for establishing feeding management strategies.

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