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FOOD ACCEPTANCE IN DIFFERENT LARVAL STAGES OF *Macrobrachium carcinus*

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ABSTRACT

Food acceptance in different larval stages of *Macrobrachium carcinus* was evaluated by investigating the stage at which the ingestion of inert moist diet food begins and by the incidence of *Artemia* nauplii and inert moist diet in the digestive tract throughout development. Two experiments were carried out: in the first study, the acceptance of the inert diet was evaluated in 60 larvae of each stage (I to XII) fed *ad libitum* after two hours of fasting. The inert diet was 100% accepted at zoea stage V. In the second study, newly hatched larva was then kept in larval tanks and fed with *Artemia* nauplii and inert moist diet simultaneously. Fifteen minutes after feeding, 50 specimens of each larval stage were examined and evaluated for the ingestion of these foods. Larvae at stage I did not feed, while larvae at stage II consumed both live and inert food. From the stage of zoea IX, the exclusive consumption of *Artemia* nauplii was not verified. These data indicate that feeding *M. carcinus* in larviculture can be initiated at stage II with inert moist diet and *Artemia* nauplii, and an exclusive supply of inert moist diet from stage IX can be recommended.

Keywords: *Artemia* nauplii; feeding behavior; larviculture; inert moist diet; prawn.

ACEITAÇÃO DE ALIMENTOS EM DIFERENTES ESTÁGIOS LARVAIS DE *Macrobrachium carcinus*

RESUMO

A aceitação alimentar nos diferentes estágios larvais de *Macrobrachium carcinus* foram avaliadas a partir da investigação da fase em que começa a ingestão de uma dieta inerte úmida e a incidência de náuplios de *Artemia* e da dieta inerte úmida no trato digestório durante todo o desenvolvimento. Foram realizados dois experimentos: no primeiro, a aceitação da dieta inerte foi avaliada em 60 larvas de cada estágio (I a XII) alimentadas *ad libitum* após duas horas de jejum. A dieta inerte úmida foi 100% aceita na fase V de zoea. No segundo experimento, larvas recém-eclodidas foram mantidas em tanques larvais e alimentadas com náuplios de *Artemia* e dieta inerte úmida simultaneamente. Quinze minutos após a alimentação, 50 espécimes de cada estágio larval foram examinados e avaliados em relação à ingestão desses alimentos. Larvas no estágio I não se alimentaram, enquanto larvas no estágio II consumiram alimento vivo e inerte. A partir do estágio de zoea IX o consumo exclusivo de náuplios de *Artemia* não foi verificado. Esses dados indicam que a alimentação de *M. carcinus* na larvicultura pode ser iniciada no estágio II, com dieta úmida inerte e náuplios de *Artemia*, e pode ser recomendado o fornecimento exclusivo de dietas úmidas inertes a partir do estágio IX.

Palavras-chave: náuplios de *Artemia;* comportamento alimentar; dieta úmida inerte; larvicultura; camarão de água doce.

INTRODUCTION

Macrobrachium carcinus stands out as a native Brazilian species of freshwater prawn and has potential as an alternative to *Macrobrachium rosenbergii* production (Valenti and Flickinger, 2020). This prawn species has high resistance to handling and variation in environmental factors, and high fecundity and fertility, with an incubation period of 19 days; it is also one of the largest Brazilian freshwater prawn species (Valenti, 2007; Lara and Wehrtmann, 2009; Kutty and Valenti, 2010). The species are exploited by artisanal fisheries and receive good acceptance in the market, making them a promising species for cultivation. Nonetheless, interest in commercial breeding of the species is scarce because techniques used in prawn production are not yet suitable for it (Kutty and Valenti, 2010).

The most cultivated freshwater prawn in Brazil is the exotic species *M. rosenbergii*. Although there is no evidence of negative impacts from the cultivation of this species, there are more advantages to using native than exotic species. Such advantages include genetic and environmental reasons, as well as increased production sustainability and local acceptance (Araujo and Valenti, 2007). For sustainable aquaculture, efforts are needed to diversify and increase the production of native species (Boyd et al., 2020). Therefore, studies with these species are necessary to develop production technology and evaluate commercial viability.

Among the factors that most affect the development and survival of organisms is food management (Yúfera and Rodríguez, 1985; Méndez-Martínez et al., 2018; Valenti and Daniels, 2000). Proper feeding results in high survival rates and high-quality larvae of freshwater prawns. Larvae of *M. carcinus* are currently fed *Artemia* nauplii during the early stages of development, while after 10 to 12 days (stage IV) this feed is supplemented with an inert moist diet. The survival rate under such a regime, however, is low, probably due to a lack of knowledge regarding the nutrition requirements of the species at this stage of development (Kutty and Valenti, 2010).

Knowledge of feeding behavior is necessary to determine appropriate food management for a particular organism (Loya-Javellana, 1989; Sorgeloos and Léger, 1992; Lavens and Sorgeloos, 2000; Aviz et al., 2018). The larvae of *M. carcinus* are small and extremely fragile during early developmental stages when they are passing through various phases with quite different morphological characteristics. Consideration of the acceptance of food throughout larval development of this species is fundamental to establishing a specific and appropriate feeding regime for successful larviculture (Loya-Javellana, 1989; Araujo and Valenti, 2017).

The present study aimed to quantitatively evaluate the feeding behavior of *M. carcinus* larvae with regards to different types of food commonly used in larviculture — *Artemia* nauplii and inert moist diet. The acceptance of inert food was assessed as a function of the larval developmental stage. The larval stage at which a higher incidence of inert food was found in the digestive tract was then determined. The resultant data are expected to define the feeding preference of larval *M. carcinus* during development and determine the most appropriate time to replace *Artemia* nauplii with inert feed.

MATERIAL AND METHODS

The study was performed at the Prawn Farming Laboratory of the Federal University of the Reconcavo of Bahia (UFRB). Larvae of *M. carcinus* were obtained from ovigerous females from river in the Municipality of Mata do São João, state of Bahia, Brazil (12°35'08.9"S - 38°02'39.5"W**).** Larval stages were identified according to Choudhury (1971).

The formulation of the inert moist diet used in the experiments was adapted from Valenti et al. (1998), and was composed of chicken egg (34.0%), mollusk cream (10.0%), fish fillet (10.0%), powdered milk (4.0%), wheat flour (2.0%), fish oil (1%), vitamin and mineral supplement l (1.4%) and water (37.6%). The ingredients were weighed, mixed in a blender, and cooked in a water bath. The feed consisted of an inert moist diet and was then divided into portions and stored in a freezer at -18ºC. The inert moist diet was passed through a stainless-steel sieve with 425 µm mesh and collected in a sieve of 250 µm before feeding the larvae. The nutritional value of this diet is approximately 40% crude protein, 24% ether extract, 23% nitrogen-free extract, 10% minerals, 20% original dry matter, and 5000 kcal kg^{-1} gross energy. The proximal analyzes of the inert moist diet was carried out at the UFRB Laboratory of Bromatology.

Two studies were carried out, each lasting 57 days: (1) acceptance of inert moist food as a function of the larval developmental stage; and (2) incidence of *Artemia* nauplii and inert moist diet in the gastrointestinal tract at different larval stages. The methodology was similar to that adopted by Araujo and Valenti (2007) for *M. amazonicum.*

Acceptance of inert moist diet as a function of larval developmental stage

The experiment was designed to determine the incidence of an inert moist diet in each of the larval stages of *M. carcinus*. The experiment was designed as a model II for a bidirectional frequency table (Sokal and Rohlf, 1995). The larval stage was one criterion and food intake, the second. Therefore, a 12 × 2 contingency table was obtained. Ten repetitions were performed for each larval stage.

A black rectangular larviculture tank containing 40L of water in salinity 20 with a heater with thermostat systems (HOPAR Aquarium Heater, Model H-606 75W - Zhongshan, Guangdong, CHINA), constant aeration, external biological filter, closed water recirculation system, and a photoperiod of 12h light and 12h dark was set up to store a stock supply of larvae at different stages of development. Salinity 20 was obtained by mixing filtered freshwater (5 µm) with seawater. A refractometer portable for salinity (INSTRUTHERM, Model RTS-101 ATC - Sao Paulo, São Paulo, BR) was used to measure the desired salinity. The seawater was transported from the Farm Oruabo, Bahia Pesca SA (Acupe, Municipality of Santo Amaro City, Bahia, Brazil). After hatching, the larvae were stocked at a density of 50 individuals L^{-1} and fed with inert moist diet twice each morning and newly hatched *Artemia* nauplii at dusk in an *ad libitum* regimen. Debris and remaining food were siphoned daily.

Water quality was monitored daily by measuring the temperature (°C) and dissolved oxygen concentration (mg L^{-1}) using a YSI, Model ProODO, instrument (Yellow Springs, Ohio, USA). Levels of total ammonia and nitrite were measured weekly using colorimetric kits (Labcon Test). The pH was measured weekly and salinity twice a week with a YSI, Model 63, instrument (Yellow Springs, Ohio, USA). Samples of larvae were periodically evaluated under a stereomicroscope (OLYMPUS, Model SZ2/SZ51–LGB - Tokyo, JAPAN) - Magnification used from 8x to 40x (depending on larval stage) to identify the larval stage. When a determined stage (zoea I to XII) was predominant in the tank, 60 larvae of the same stage were separated and transferred to ten 150-mL glass vials containing 100 mL of water at a salinity of 20 (six larvae/flask). The water used in the flasks came from the larviculture tank and was filtered through a 125 µm nylon mesh screen. The glass vials were visually isolated from each other by a black protective film and placed on a white tray containing

water and a heater with thermostat to maintain the temperature constant. Each glass vial was provided with sufficient aeration to keep the inert moist diet in suspension.

After a two-hour fasting period, the larvae were fed the inert moist diet *ad libitum*. The larvae were withdrawn from the vials after 15 minutes in contact with the food using a 3 mL graduated Pasteur pipette. The larvae were then placed in Petri dishes for analysis under an optical (OLYMPUS, Model SZ2/SZ51–LGB - Tokyo, JAPAN) - Magnification used from 8x to 40x (depending on larval stage) to determine the incidence of an inert moist diet in the digestive tract. The gastrointestinal content of each larva was classified as either (a) absence of food (stomach and intestine empty), or (b) presence of food (stomach and intestine partially or fully occupied). The frequency of acceptance of the inert moist diet was calculated as the percentage of larvae observed (60 larvae) at a given larval stage with food in the gastrointestinal tract.

Incidence of *Artemia* nauplii and inert moist diet in the gastrointestinal tract at different larval stages

The experiment was designed to determine any preference for ingesting *Artemia* nauplii or inert moist diet during the development of larval *M. carcinus*. The experiment was designed as model II for a two-way frequency table (Sokal and Rohlf, 1995). The larval stage was one criterion, and the ingested food was the second one. As ingested food was classified into four categories, a 12×4 contingency table was obtained. For each larval stage, 50 larvae were sampled and analyzed.

Newly hatched larvae were stored at a density of 50 individuals L^{-1} in a rectangular black tank containing 40 L of water (salinity 20), with conditions and management as described for the previous experiment.

To analyze food incidence in the digestive tract the tank was siphoned in the morning before supplying food to the larvae to remove the remaining food offered the night before. After a fasting period of two hours, the larvae were fed simultaneously inert moist diet (same as used in the first experiment) and newly hatched *Artemia* nauplii. Fifteen minutes after feeding, larvae were randomly captured in the tank using a 3 mL graduated Pasteur pipette and placed in Petri dishes for individual observation under an optical stereomicroscope (OLYMPUS, Model SZ2/SZ51–LGB - Tokyo, JAPAN) - Magnification used from 8x to 40x (depending on larval stage). After confirming the predominant developmental stage (zoea I to XII) of 50 larvae, the food incidence (FI) of each food type in the digestive tract was evaluated. The ingested food was distinguished by coloration, with *Artemia* being orange and inert moist diet yellow (Figure 1).

Ingested contents were classified as (A) only *Artemia* nauplii; (B) only inert moist food; (C) *Artemia* and inert moist diet; and (D) no food. Food incidence was calculated for each larval stage as the percentage of the total number of larvae observed (50 larvae) that had the same classification of ingested content.

In the experiments, larval frequencies in each food category eaten by larval stages were compared by the Chi-square independence test (Sokal and Rohlf, 1995). All analyzes were performed using the software "Statistical Analysis System - SAS" (version 9.4) and the significance level was set at $p < 0.05$.

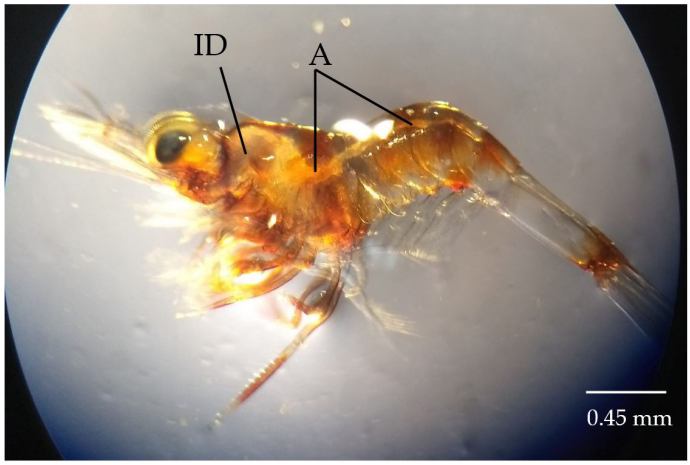

Figure 1. *Macrobrachium carcinus* larvae in stage VIII with *Artemia* (A-orange) and inert moist diet (ID-yellow) in the digestive tract. (Magnification: 20x).

RESULTS

The water quality of the larval stock remained within recommended levels during the two experiments (Valenti et al., 2010). Mean values (± standard deviation) of the water quality parameters of the larval stock tank were: temperature 29.7 ± 0.5°C; pH 8.00 ± 0.05; dissolved oxygen 5.80 ± 0.37 mg L^{-1}; and ammonia and nitrite concentrations between 0.00 and 0.25 mg L^{-1}.

The first experiment found a significant difference ($p < 0.05$) in the acceptance of the inert moist diet during larval development. Acceptance of the inert moist diet was observed beginning at zoea stage II, for which 63% of the larvae had inert food in the digestive tract. This percentage increased gradually, reaching 100% at stage V (Figure 2).

Feeding incidence (FI) in the digestive tract of *M. carcinus* varied significantly according to the food offered ($p < 0.05$). An empty digestive tract was observed in stage I. Ingestion of both

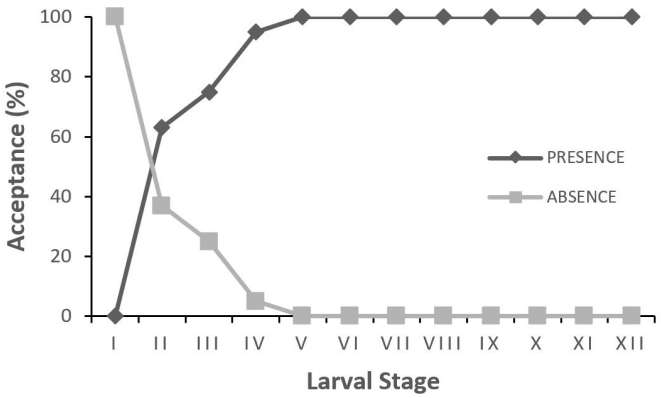

Figure 2. Presence of inert moist diet in the digestive tract of *Macrobrachium carcinus* as a function of larval stage. Absence = empty; Presence = digestive tract partially or fully occupied.

foods began to be detected at zoea stage II, for which 42% of the larvae fed on *Artemia* nauplii and only 14% on the inert moist diet. The consumption of inert moist diet increased to 30% in stage III, however, there was no statistical difference concerning the consumption of *Artemia* nauplii (28%). There was a progressive increase in the exclusive consumption of inert moist diet from stage IV, with a significant difference (p <0.05) between consumption of *Artemia* nauplii. There was an increase in the acceptance of the two types of food (*Artemia* and inert moist diet) and these differed significantly (p <0.05) between exclusive consumption of the inert moist diet from stage VII. As of stage IX, there was no exclusive consumption of *Artemia* nauplii, and the consumption of inert moist diet became more important than the consumption of live food (Figure 3).

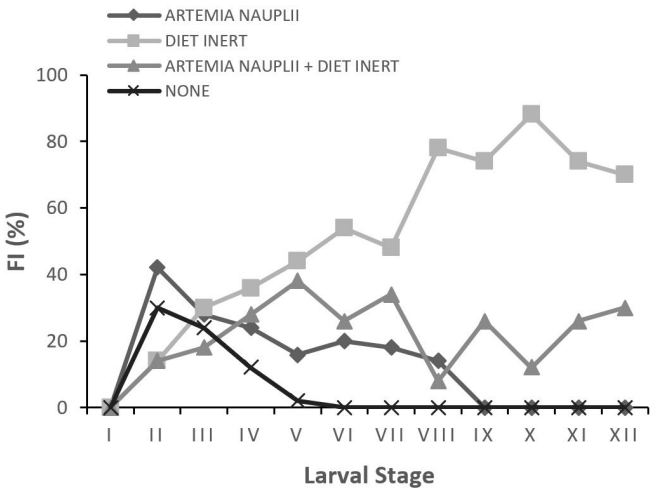

Figure 3. Food incidence (FI) in the digestive tract of *Macrobrachium carcinus* in each larval developmental stage in relation to an inert moist diet and *Artemia* nauplii.

DISCUSSION

Data from both experiments revealed that none of the larvae at stage I fed. Similar results were reported for *M. rosenbergii* by Barros and Valenti (1997) and for *M. amazonicum* by Araujo and Valenti (2007). According to these authors, larvae in stage I grasp the food particles that touch their thoracic appendages (maxillipeds), however, ingestion does not occur, possibly due to the larvae possessing a large amount of yolk reserve in the early stages (Araujo and Valenti, 2011).

Both foods were ingested by larval *M. carcinus* beginning at stage II, which was confirmed by visualization of *Artemia* and/or inert moist diet in the gastrointestinal tract of about 60 to 70% of the larvae. Based on these data, it can be inferred those larvae of this species have a greater capacity to take advantage of the food available in the water body beginning in the first larval stages, and are possibly omnivorous throughout the larval cycle, and not passing through an exclusively carnivorous phase as do *M. amazonicum* and *M. rosenbergii.* Studies aiming at characterizing ontogenetic stages concerning the morphology of the digestive system of *M. carcinus* may corroborate this hypothesis.

The data also demonstrate a greater ability of *M. carcinus* larvae to capture and ingest available food in the early stages of development, and to suggest a higher nutritional need of the larvae of this species. These findings thus corroborate Coelho-Filho et al. (2018), who found that larvae of *M. carcinus* up to zoea stage X consume a greater quantity of *Artemia* nauplii than do larvae of *M. rosenbergii*. Barros and Valenti (2003) verified that larvae of *M. rosenbergii* only accept an inert diet beginning at stage VII. In other words, larvae of *M. carcinus* have a greater need to ingest *Artemia* nauplii and feed more early on inert moist diet than larvae of *M. rosenbergii*, demonstrating that they have a different feeding behavior.

In the first experiment of the present study, ingestion of inert moist diet by larvae (63%) was observed beginning at stage II of development. However, the second experiment found that when live food and inert moist diet were offered simultaneously at stage II, the larvae better accepted *Artemia* nauplii (40%) than inert moist diet (14%). This finding can be explained by the difference in the volume of water used in the two experiments or perhaps by a preference for live food when present in the medium.

The lower volume of water used (100 mL) in the first experiment may have provided greater contact between larvae and particles of the inert moist diet, thus increasing the opportunity for capture and ingestion. The larvae of decapod crustaceans are usually passive consumers, depending on chance encounters to capture and ingest prey (Barros and Valenti, 2003). Due to the small volume of water, the inert food remained in the water column for a longer period, and in constant movement due to the aeration, thus increasing the chance of encounter and ingestion by larvae. Another hypothesis for the greater consumption of inert moist diet in the first experiment is the absence of another food source in the medium, which in this case was *Artemia* nauplii. When available simultaneously, there was a clear preference for live food at this stage (stage II).

Beginning with stage III, the ingestion of inert moist diet exceeded that of *Artemia* nauplii when supplied simultaneously, and in stage VI more than 50% of the larvae exclusively ingested inert moist diet. Although there was still simultaneous ingestion of both types of food, the consumption of *Artemia* decreased with larval development while that of the inert moist diet increased. These results can be explained by changes in the perception capacity of the food in the medium, as well as the morphophysiological characteristics of larvae during development. Throughout development, larvae of species of *Macrobrachium* develop mouthparts (Rocha et al., 2016, 2018), antennules, endopodites with chemoreceptors of smell and touch (Ache, 1982). With the development of these structures, mechanoreception ceases to be the main mechanism to distinguish the presence of food in the environment. Throughout development, larvae of *M. carcinus* may be able to detect food more easily and swim towards it. Another explanation may be the search for biochemical diversity

and food availability in the medium, or the greater attractiveness of the inert moist diet compared to *Artemia* nauplii at stage III.

In the present study, starting at stage IX the larvae of *M. carcinus* did not exclusively ingest *Artemia* nauplii and the consumption of inert moist diet became more important than the consumption of live food. According to Coelho-Filho et al. (2018), the consumption of *Artemia* nauplii by this species halves decreases at stage IX. This is possibly related to the difficulty in catching live food compared to inert food.

According to Dhont et al. (2010), prey size and morphology determine ingestion efficiency. According to Barros and Valenti (2003), capture efficiency decreases when this ratio decreases. The best ratio of the larval size of *M. carcinus* to that of prey (*Artemia*) was 0.12 (Coelho-Filho et al., 2018), while the best ratio for *M. rosenbergii* was estimated to be about 0.20 (Barros and Valenti, 2003).

To increase ingestion of live food by larvae, it is necessary to supply it in greater quantity, however, this practice is not recommended for commercial cultivation due to the high cost of *Artemia* cysts, suggesting supplementation with inert moist diets, mainly beginning at stage IX for *M. carcinus* (Coelho-Filho et al., 2018). However, Santos et al. (2007) found good results for larviculture of *M. carcinus* when the formulated diet was combined with *Artemia* nauplii beginning at stage VI.

CONCLUSION

In conclusion, feeding of *M. carcinus* larvae should begin at stage II with both inert moist diet and *Artemia* nauplii, and then at stage IX, the only inert moist diet should be offered. The reduce number of *Artemia* nauplii supplied throughout development can reduce productions and avoid nauplii that are not ingested by larvae increase organic matter in the medium, which damages the water quality of the culture. Further studies may confirm this hypothesis.

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