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ASPECTS OF FOOD MANAGEMENT ON AMAZON RIVER PRAWN LARVICULTURE PHASE*

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ABSTRACT

The time supply of live food (*Artemia* nauplii) at the initial phases of development and the frequency of feeding with inert feed (egg custard) in *Macrobrachium amazonicum* larviculture were evaluated by two experiments: In Experiment I, newly hatched *Artemia* were offered to the larvae in three schedules (treatments): at 07:30 h (A07:30); at 12:00 h (A12:00) and 16:30 h (A16:30). In Experiment II, the inert food was offered in the following frequencies (treatments): twice a day - at 08:00 and 17:00 h (IF2); three times a day - at 08:00; 12:30 and 17:00 h (IF3) and four times a day - at 08:00; 11:00, 14:00 and 17:00 h (IF4). Water quality variables (dissolved oxygen, pH, temperature, salinity, NH_3 + NH_4, NO_2 and NO_3) and production variables (weight, survival and duration of larviculture) were evaluated. The feeding managements studied did not influence significantly either the water quality and production variables. The results indicated that it can be recommended, for *M. amazonicum* larviculture feeding management, the supply of *Artemia* nauplii at early morning and inert feed two times per day (early morning and late afternoon), after stage V until the metamorphosis.

Key words: frequency of feeding; live food; *Artemia* nauplii; inert food.

MANEJO ALIMENTAR NA LARVICULTURA DO CAMARÃO-DA-AMAZÔNIA

RESUMO

Avaliou-se o horário de fornecimento de alimento vivo no início do desenvolvimento larval e a frequência de arraçoamento com alimento inerte em larvicultura *Macrobrachium amazonicum*. Foram realizados dois experimentos: no Experimento I, náuplios de *Artemia* (A) foram ofertados em três horários (tratamentos): às 07:30 h (A7:30); às 12:00 h (A12:00) e 16:30 h (A16:30). No Experimento II, o alimento inerte (AI) foi oferecido nas seguintes frequências (tratamentos): duas vezes ao dia - às 08:00 e 17:00 h (AI2); três vezes ao dia - às 08:00; 12:30 e 17:00 h (AI3) e quatro vezes ao dia - às 08:00; 11:00, 14:00 e 17:00 h (AI4). Foram avaliadas as variáveis limnológicas (oxigênio dissolvido, pH, temperatura, salinidade, NH_3+NH_4, NO_2 e NO_3) e as variáveis de produção (peso, sobrevivência e dias de larvicultura). Os diferentes manejos avaliados não influenciaram significativamente na qualidade da água e nas variáveis de produção. Os resultados indicaram que, para o manejo alimentar na larvicultura de *M. amazonicum*, o alimento vivo (náuplios de *Artemia*) pode ser fornecido no início da manhã e, após o estágio V, a dieta pode ser complementada com o alimento inerte, duas vezes por dia (início da manhã e final da tarde).

Palavras-chave: frequência de alimentação; alimento vivo; náuplio de *Artemia*; alimento inerte.

INTRODUCTION

After alternating periods of decreasing and expansion, the freshwater prawn farming in Brazil currently presents a favorable scenario, due to the prospects of improvement of the organization of the productive chain (MARQUES and MORAES-VALENTI, 2012). The only commercially farmed species in Brazil is *Macrobrachium rosenbergii* (DE MAN, 1879), but native species as *Macrobrachium amazonicum* (HELLER, 1862), *M. acanthurus* (WEIGMANN, 1836) and *M. carcinus* (LINNAEUS, 1758) are exploited by fishing in the north and northeast regions (KUTTY and VALENTI, 2010). Among these species, *M. amazonicum* presents great potential for aquaculture (MACIEL and VALENTI, 2009), being used, besides human consumption, as ornamental species, live feed for carnivorous ornamental fish and live bait for sport fishing (MARQUES and

MORAES-VALENTI, 2012). Although the larviculture techniques of the species are well developed, feeding management in the different larval stages still presents controversial results.

In order to establish feeding management, the behavior of the larvae facing the diet supplied should be known. Behavior studies have mainly evaluated the dependence of exogenous food on larval development (ANGER and HAYD, 2009, 2010); acceptance of inert food according to the stage of development (ARAUJO and VALENTI, 2007); ingestion of *Artemia* according to stage of development, supply density of nauplii and period of day (MACIEL *et al*., 2012) and the influence of the color of the larvae maintenance tanks in the food intake (MACIEL and VALENTI, 2014a). Also the replacement of *Artemia* nauplii by inert food was investigated (MACIEL and VALENTI, 2014b; ARAUJO and VALENTI, 2017).

Despite these studies, some aspects of feeding management need to be better investigated, as the feeding time with live food during the initial larval stages and the frequency of feeding with inert feed (custard). Those aspects can influence the food intake, it's assimilation by the larvae and, consequently, larval development and productivity.

Macrobrachium amazonicum larvae feed on *Artemia* nauplii from the second stage of larval development (ARAUJO and VALENTI, 2007), with the recommended frequency of once a day MACIEL *et al*. (2012). Additionally, these authors comment that is necessary establishing the best time of the supply of nauplii, considering the feeding activity of the prawn larvae.

Regarding to inert feed supply, ARAUJO and VALENTI (2007, 2017) recommend starting feeding *M. amazonicum* larvae from stage VI, as a complement to the feeding with *Artemia*, considering that since stage VII, inert feed becomes to be more consumed than live food. The larger availability of inert feed appears occurring just after the supply, because even with aeration, the flakes of feed rapidly precipitate on the bottom, becoming less accessible for the larvae, which have planktonic behavior. Thus, the unconsumed feed can be microbiologically decomposed resulting in an increase of nitrogen compounds, thus prejudicing the water quality. Additionally, after some time in the water, there is leaching of the feed nutrients. Consequently, the supply of inert feed in two or more portions during the daytime could increase the chance encounter of the larvae with the feed, minimizing the wastes and improving the absorption of nutrients by the larvae (MACIEL, 2007).

Considering these aspects, the objective of this study was establishing a protocol aiming to maximize the feeding efficiency on *M. amazonicum* larviculture, determining the most adequate time for *Artemia* supply in the initial larval phase and the frequency of inert feed supply after stage V.

METHODS

Two experiments were carried out during January to March, 2015 at the Freshwater Prawn Laboratory of the Pirassununga Aquaculture Centre. The general procedures, common for both experiments were described below and after that, the specific procedures to experiments I and II were presented.

Larvae of *M. amazonicum* prawns were obtained from ovigerous females collected in a breeding pond and transferred to a hatching tank of 100L, inside the laboratory. After hatching, larvae were counted and transferred to the experimental tanks (rectangular plastic 30 L black boxes, with 20 L of useful volume) with water recirculation system (external biological filter), at a density of 50 L^{-1}. The recirculation rate was maintained at 100% of total volume per hour. Starting 14 days before the beginning of the experiments, the biofilters were gradually activated, adding ammonium (NH_3) solution to them.

The photoperiod was 12 h clear / 12 h dark. The wastes of food, exoskeletons, dead larvae and feces of the experimental tanks were syphonated daily, in order to keep good water quality. Temperature, dissolved oxygen (YSI Pro 20 oxymeter), pH (EcoSense 100A pH meter) and salinity (INCOTHERM Salinometer), were measured once a day (from 8:00 to 09:00 a.m.), and were maintained in the appropriate range for *M. amazonicum* larvae (ARAUJO and VALENTI, 2011; HAYD *et al*., 2014). In alternate days, the monitoring of ammonium (NH_3), nitrite (NO_2) and nitrate (NO_3) concentrations inside the tank was determined by colorimeters kits for marine aquarium (PRODAC tests).

Samples composed by 10 larvae of each tank were daily collected and analyzed using a stereomicroscope, in order to identifying the average larval stage, according to the methodology described by GUEST (1979).

Experiment 1: determination of best timing for live food supply (*Artemia* nauplii) in the initial larviculture phase (II-VI stages)

In Experiment 1, from stage II, larvae were fed only with newly hatched *Artemia* nauplii, at 07:30 h, 12:00 h and 16:30 h (A7:30, A12:00 and A16:30 treatments respectively). The experiment followed a complete randomized design, with four replicates per treatment. The amount of *Artemia* supplied was 4 nauplii mL^{-1} in stage II and 6 nauplii mL^{-1} in stages III to VI (MACIEL and VALENTI, 2014a).

Water quality variables (Mean ± SD) for the treatments were: temperature: A7:30 = 29.4 ± 0.7 °C; A12:00 = 28.8 ± 0.4 °C, A16:30 = 29.6 ± 0.9 °C; dissolved oxygen: A7:30 = 4.64 ± 0.40 mg L-1; A12:00 = 5.19 ± 0.35 mg L^{-1}; A16:30 = 5.03 ± 0.09 mg L^{-1}; pH: A7:30 = 7.90 ± 0.15; A12:00 = 8.02 ± 0.04; A16:30 = 7.92 ± 0.05; salinity: A7:30 = 13.7 ± 0.8 g L^{-1}; A12:00 = 13.8 ± 0.8 g L^{-1}; A16:30 = 13.6 ± 1.1 g L^{-1}.

Larvae were harvested when more than 80% of the population of each tank reached the stage VI (estimated by random sampling). The filtering system was turned off, the water drained and the larvae were individually counted for estimating the survival of each tank.

The dry mass of the larvae was obtained from random samples of 200 larvae from each tank, separated into 10 subsamples with 20 individuals. Each subsample was packed into pre-weighed aluminum foil cartridges (initial mass) and identified according

to each treatment. These were then placed in Petri dishes and dried in an electric kiln at 60 °C for 12 hours; the plates were transferred to a desiccator for 2h, and after, each cartridge was weighed (final mass) in an analytical balance Mettler Toledo XP26 (1 mμ precision). The dry mass was determined by the difference between the final mass and the initial mass of the cartridges, according to the treatments.

Experiment 2: determination of the best frequency of inert feed supply (egg custard) on the final phase of *M. amazonicum* larval development (stages V-IX)

In Experiment 2, inert food was offered in the following frequencies (treatments): twice a day - at 08:00 and 17:00 h (IF2); three times a day - at 08:00; 12:30 and 17:00 h (IF3) and four times a day - at 08:00; 11:00 h, 14:00 and 17:00 h (IF4). The experiment was conducted on complete randomized design, with four replicates per treatment.

The inert food consisted of the "egg custard", adapted from the supplementary diet for *M. rosenbergii* larvae, described by MALLASEN and VALENTI (1998). The ingredients were weighed, blended and the resulting mixture was cooked in a pan under water bath to custard consistency. After cooling, it was cut into small pieces, individually wrapped with polyethylene film and frozen at −18 °C. Before being fed to the larvae, the pieces were made into smaller particles, which were then passed through a sieve with 1.00 mm mesh and the filtrate was collected in a 0.5 mm mesh screen for larvae on stages V and VI, and in a 0.7 mm mesh screen for stages VII onward.

Larvae were feed on the basis of the procedures adopted by MACIEL and VALENTI (2014a), consisting on newly hatched *Artemia* supplied daily at 10:00 h, from the second larval stage onwards, and inert food supplied from stage VI onwards, according to the treatments described above. The daily amount supplied of inert food was the same for all treatments, varying of 0.5 g $tank^{-1}$ at stages VI and VII and 1.0 g $tank^{-1}$ from stage VIII onwards.

Water quality variables (Mean ± SD) for the treatments were: AI2 = 28.0 ± 0.4 °C; AI3 = 28.4 ± 0.4 °C; AI4 = 27.9 ± 0.9 °C (temperature); AI2 = 5.66 ± 0.12 mg L^{-1}, AI3 = 5.65 ± 0.14 mg L^{-1}, AI4 = 5.80 ± 0.17 mg L^{-1} (dissolved oxygen); AI2 = 7.90 ± 0.15; AI3 – 8.02 ± 0.04, AI4 = 7.92 ± 0.05 (pH) and AI2 = 13.7 ± 0.8 g L^{-1}, AI3 – 13.8 ± 0.8 g L^{-1}, AI4 – 13.6 ± 1.1 g L^{-1} (salinity).

The tanks were harvested when more than 80% of the larvae population metamorphosed into post-larvae (PL) (estimated by random sampling). All the PL and the remaining larvae were counted in order to calculate survival rate. The dry mass for each experimental tank was determined as described for the larvae (experiment I). After that, the means of production variables with their respective standard deviations (survival rate, dry mass and duration of larviculture) were calculated to the each treatment.

Statistical analysis

Before the hypothesis test, the survival data were square-root arc-sine transformed as recommended for ZAR (2010), but they are presented as non-transformed percentages for easier visualization.

Data of nitrogenous compounds and production were tested for normality (Shapiro-Wilk) and homoscedasticity (Bartlett). As no significant deviation was observed, means were subjected to one-way ANOVA, using Statistica version 7.0. When differences among variables were observed, the means were compared by the Tukey Test ($p<0.05$).

RESULTS

Experiment 1

Apparently, the *Artemia* supplying time has not significantly influenced the concentration of ammonium (NH_3), nitrite (NO_2) and nitrate (NO_3) (Table 1). However, the high standard deviations may have masked possible effects of treatments on such variables. Lower concentrations were observed on A7:30 treatment and the higher, on A16:30 treatment.

Production variables (dry mass, survival rate, and days of larviculture) did not differ statistically ($p<0.05$) among treatments, whereas survival rate presented high SD values in the treatments A12:00 and A16:30 (Table 2).

Experiment 2

As in the Experiment 1, water variables total ammonium ($NH_3 + NH_4$), nitrite (NO_2) and nitrate (NO_3) were not significantly influenced ($p<0.05$) by the frequencies of inert feed supplying (Table 3).

Table 1. Water quality variables (mean ± SD) total ammonium ($NH_3 + NH_4$), nitrite (NO_2) and nitrate (NO_3), according to the treatments tested in Experiment 1: A7:30 - *Artemia* supplied at early morning; A12:00 - *Artemia* supplied at noon; A16:30 - *Artemia* supplied at late afternoon.

Variable	Treatment		
	A7:30	A12:00	A16:30
$NH_3 + NH_4$ (mg L^{-1})	0.29 ± 0.36	1.04 ± 1.34	1.18 ± 1.61
NO_2 (mg L^{-1})	0.02 ± 0.03	0.18 ± 0.33	0.72 ± 1.21
NO_3 (mg L^{-1})	4.96 ± 3.47	5.06 ± 4.87	5.25 ± 1.66

Table 2. Production variables (mean ± SD) dry mass (DM), survival rate (SR) and days of larviculture (DL) (Mean ± SD), according to the treatments tested in Experiment 1: A7:30 - *Artemia* supplied at early morning; A12:00 - *Artemia* supplied at noon; A16:30 - *Artemia* supplied at late afternoon.

Variable	Treatment		
	A7:30	A12:00	A16:30
DM (mg)	1.99 ± 0.47	2.09 ± 0.53	2.26 ± 0.40
SR (%)	74.8 ± 3.5	61.7 ± 11.1	67.3 ± 14.3
DL (days)	8.3 ± 1.3	7.5 ± 1.3	7.5 ± 1.7

Table 3. Water quality variables (mean ± SD) total ammonium (NH_3 + NH_4), nitrite (NO_2) and nitrate (NO_3), according to the treatments tested in Experiment 2: IF2 - inert feed supplied two times per day (at 08:00 and 17:00 h); IF3 - inert feed supplied three times per day (at 08:00; 12:30 and 17:00 h) and IF4 - inert feed supplied four times per day (at 08:00; 11:00 h, 14:00 and 17:00 h).

Variable	Treatment		
	IF2	IF3	IF4
NH_3 + NH_4 (mg L^{-1})	0.05 ± 0.04	0.04 ± 0.04	0.00 ± 0.00
NO_2 (mg L^{-1})	0.67 ± 1.22	0.07 ± 0.04	0.09 ± 0.04
NO_3 (mg L^{-1})	3.80 ± 0.59	3.36 ± 1.32	4.08 ± 2.04

Table 4. Production variables (mean ± SD) dry mass (DM), survival rate (SR) and days of larviculture (DL) (Mean ± SD), according to the treatments tested in Experiment 2:-IF2 - inert feed supplied two times per day (at 08:00 and 17:00 h); IF3 - inert feed supplied three times per day (at 08:00; 12:30 and 17:00 h) and IF4 - inert feed supplied four times per day (at 08:00; 11:00 h, 14:00 and 17:00 h).

Variable	Treatment		
	IF2	IF3	IF4
DM (mg)	11.15 ± 0.78	11.09 ± 1.04	11.68 ± 2.58
SR (%)	42.4 ± 13.0	52.5 ± 8.4	47.6 ± 10.0
DL (days)	20.3 ± 1.0	19.8 ± 1.0	20.5 ± 1.7

Production variables did not present statistical differences ($p<0.05$) among the treatments. However, means of survival rates showed high standard deviation (Table 4).

DISCUSSION

The concentration of nitrogen compounds remained at the recommended range to this species (<1.6 mg L^{-1} for ammonium, <0.8 mg L^{-1} for nitrite and <80.0 mg L^{-1} for nitrate), according to MORAES-VALENTI and VALENTI (2010). Nitrogen by-products are resulting from both larvae excretion and decomposition of organic matter especially wastes of food. Considering that the food management can influence the food intake by the larvae, it can be inferred that the biofiltration system was not overloaded due to the unconsumed food, indicating that the managements evaluated did not negatively affect the nitrification process and could be used on *M. amazonicum* larviculture.

However, it is important to highlight that the high standard deviations observed in the concentration of nitrogen compounds of both experiments, could indicate the smaller or the higher adjusting to the feed management in larval phase. The higher the intake by larvae, the smaller the waste of feed and, consequently, minor variation in concentration of nitrogen compounds is expected.

During the initial larval development of *M. rosenbergii*, the sensorial and food apprehension structures are barely developed (HENRIQUES *et al*., 2014). Consequently, the larvae are not able to efficiently pursuit their prey. According to BARROS and VALENTI (1997), this characteristic makes the chance of encounter is the main mechanism of gathering food in earlier larval stages. Indeed, MACIEL *et al*. (2012) verified that for *M. amazonicum* larvae, the encounter opportunity is the most important factor of gathering food during the night whereas the vision is the most important mechanism under day light, making that the supply of feed is more easily encountered by larvae along the day. Based on the results here obtained, it can be recommended, for *M. amazonicum* larviculture feeding management, the supply of *Artemia* nauplii once a day at early morning, aiming to reduce labor costs.

The survival rates registered in the second experiment, were higher than that presented for MURTHY *et al*. (2008) (between 31.1 and 38.9%), that studied different formulated inert larval diets. DAVID *et al*. (2016), studying the effects of intensification on the *M. rosenbergii* larviculture, obtained survivals between 55.0 ± 5.7% and 63.4 ± 9.6%, but it must be considered that the *M. amazonicum* larviculture protocol is not so well studied as *M. rosenbergii* one, and survival rates can oscillate among different cycles and may be sound improved with further studies.

The supply of inert diet twice a day is generally used both for *M. rosenbergii* (DAVID *et al*., 2016) and *M. amazonicum* (MACIEL and VALENTI, 2014b) larvae. In a recent experiment, ARAUJO and VALENTI (2017) found that productivity and survival were significantly higher when *M. amazonicum* larvae was fed twice a day with inert food and *Artemia* during all the larval stages, than when larvae were fed only with inert food four times per day along the entire cycle.

KOVALENKO *et al*. (2002) that studied a semi-purified microbound diet containing alginate was compared to newly hatched live *Artemia* nauplii as an exclusive diet for the culture of larval freshwater prawn *M. rosenbergii*. Those authors fed the larvae with the inert diet several times during the light phase (every 1.5–2 h) and obtained survival rates varying from 77.3% to 73.3%. The survivals were not significantly different from that of *Artemia*-fed larvae. Contrasting to those results, due to the lack of statistical differences among the treatments in the second experiment, it can be recommended the supply of inert feed only twice a day (treatment IF2) after stage V, until the metamorphosis into post-larvae instead of three or four times (treatments IF3 and IF4), in order to reduce costs with labor.

CONCLUSION

Once the adjustment of feeding routine is one of the most important factors in crustacean larviculture, based on the results obtained in the present study, it can be recommended, for *M. amazonicum* larviculture feeding management, the supply of *Artemia* nauplii once a day at early morning and inert feed two times per day (at early morning and late afternoon), after stage V until the metamorphosis.

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