ASSESSING THE POTENTIAL OF PARTIAL REPLACING OF *Artemia* BY PRACTICAL INERT DIET IN THE LARVICULTURE OF THE AMAZON RIVER PRAWN*

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ABSTRACT

The aim of this study was to evaluate the effect of the replacement of *Artemia* nauplii by egg-custard in the final phase of *Macrabrachium amazonicum* hatchery. The treatments were: AN-IX – larvae were fed *Artemia* nauplii during all culture and when they reached stage V, inert diet was added to the feed (control); AN-VII – larvae were fed *Artemia* nauplii from stage II to stage VI and it was totally replaced by inert diet from stage VII onwards; AN-V – larvae were fed *Artemia* nauplii from stage II to stage IV and it was totally replaced by inert diet from stage V onwards. Larvae were reared in 120-L culture tanks, coupled to biological filters. Larvae largely ingested inert diet when it was the single food available (90-100% of animals showed digestive tract full). Nonetheless, feeding larvae of *M. amazonicum* with solely inert diet from stage V or VII onward, negatively affect larval development and survival. Productivity drastically decreased from ~60 PL L^{-1} (control) to ~40 PL L^{-1} in AN-VII and ~10 PL L^{-1} in AN-V. The dry mass of the post-larvae declined ~25% in the AN-V and AN-VII. This may be due to low digestibility or nutritional deficiency of the inert diet. Therefore, the complete replacement of *Artemia* nauplii until stage VII is not recommended for the management of Amazon river prawn larviculture.

Keywords: *Macrobrachium amazonicum;* egg-custard feeds; prawn larvae

POTENCIAL USO DA DIETA INERTE COMO UM SUBSTITUTO PARCIAL AOS NÁUPLIOS DE *Artemia* NA LARVICULTURA DO CAMARÃO-DA-AMAZÔNIA

RESUMO

O objetivo desse estudo foi avaliar o efeito da substituição total dos náuplios de *Artemia* pela dieta inerte (creme de ovos) na fase final da larvicultura do *Macrobrachium amazonicum*. Os tratamentos adotados foram: NA-IX – larvas alimentadas com náuplios de *Artemia* durante todo cultivo e oferta da dieta inerte a partir do estágio V (controle); NA-VII – larvas alimentadas com *Artemia* até o estágio VI e somente dieta inerte a partir do estágio VII; NA-V alimentadas com *Artemia* até o estágio IV e somente dieta inerte a partir do estágio V. Os cultivos foram realizados em tanques de 120 L acoplados a filtros biológicos. As larvas ingeriram dieta inerte, sendo observado o preenchimento do tubo digestório, em 90 a 100% dos animais, quando este foi o único alimento ofertado. No entanto, a substituição dos náuplios de *Artemia* a partir dos estágios V e VII afetaram negativamente o desenvolvimento larval e a sobrevivência. A produtividade caiu drasticamente de ~60 PL L^{-1} (controle) para ~40 em NA-VII e ~10 em NA-V. As pós-larvas sofreram redução de massa em ~25% em NA-VII e NA-V, em relação a NA-IX. Isso deve ser devido à baixa digestibilidade ou carência nutritiva da dieta inerte. Portanto, a substituição total da *Artemia* até o estágio VII não é recomendada para o camarão-da-amazônia.

Palavras chave: *Macrobrachium amazonicum*; creme de ovos; larva de camarão.

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INTRODUCTION

The Amazon river prawn *Macrobrachium amazonicum* is widely distributed in South America (HOLTHUIS, 1952; MELO, 2003; VALENCIA and CAMPOS, 2007). This species is exploited by artisanal fisheries in the Brazilian North and Northeast and is the most abundant prawn in the Amazon region (VALENTI and MORAES-RIODADES, 2004; MACIEL and VALENTI, 2009). The Amazon river prawn is readily marketed in Brazil, although the fisheries production is insufficient to meet the growing demand (SILVA *et al*. 2007). Technological aspects of its culture are currently under research to develop a commercial farming system (MORAES-VALENTI and VALENTI, 2010).

Raising the larvae is the most complex phase of the prawn farming process (VALENTI and DANIELS, 2000). Adequate diet is essential and feed acceptance as well as nutrient requirements may change during larval development (BARROS and VALENTI, 2003). *Macrobrachium amazonicum* has nine larval stages (GUEST, 1979) and metamorphosis is completed in ~20 days in aquaculture tanks (MORAES-VALENTI and VALENTI, 2010). During the first stage, larvae are primarily lecithotrophic (ANGER *et al*., 2009; QUEIROZ *et al*., 2011) and consume the yolk. Exogenous feeding take place from stage II onwards, when larvae can be raised fed on *Artemia* nauplii and inert diet (ARAÚJO and VALENTI, 2007).

Artemia nauplii are used as the primary dietary input for the larviculture of a wide range of aquatic species. Nevertheless, the production of *Artemia* cysts fluctuates considerably in response to variations in environmental conditions, which may affect their availability, and nutritional quality of the nauplii (LAVENS *et al*., 2000; DHONT *et al*., 2010). These fluctuations in production together with variations in demand result in considerable oscillation in supplies, which are reflected in the price of the cysts. This item may attain about 30% of the total production costs of the freshwater prawn *Macrobrachium rosenbergii* post-larvae (LAVENS *et al*., 2000). Thus, the development of an effective alternative diet to replace *Artemia* during at least one phase of the larviculture, would contribute to reduce production costs.

ARAÚJO and VALENTI (2007) found that *M. amazonicum* larvae accept inert diet (egg-custard) from stage IV onwards, and shows a marked preference for this food from stage VII on. MACIEL and VALENTI (2012) feeding *M. amazonicum* larve with *Artemia* nauplii plus inert diet have observed that the higher consumption of *Artemia* is not associated with the higher productivity. OHS and D'ABRAMO (1998) tested a diet processed in a spray-dryer and KOVALENKO *et al.* (2002), a micro-agglutinated food to replace *Artemia* in feeding *M. rosenbergii* with promising results. Thus, we hypothesized that it is possible to replace live food in a phase of the *M. amazonicum* larviculture by using egg custard, a practical diet traditionally used in *Macrobrachium* hatcheries. Although practical diets show variable nutrient composition, the same occurs with *Artemia* nauplii (DHONT *et al*., 2010). Partial replacement of live food may be very important for the development of more cost-effective technology for the production of Amazon river prawns. Therefore, the aim of the present study was to test the effect of the complete replacement of *Artemia* nauplii by egg custard in the final phase of *M. amazonicum* hatchery on larvae development and post-larvae production.

MATERIAL AND METHODS

Origin of the Animals and Experimental Conditions

The study was conducted in the Crustacean Sector of Aquaculture Center (CAUNESP) at São Paulo State University, Jaboticabal, São Paulo. Larvae were obtained from a broodstock originated from wild prawns collected in northeast Pará state, Brazil (1°13'25"S, 48°17'40"W), in 2001.

The larvae were collected, counted, acclimatized, and stocked (80 larvae L^{-1}) in 120-L black cylindrical culture tanks, coupled to biological filters in a recirculating system described in VALENTI *et al*. (2009). Thermostatically controlled heaters were installed to maintain the water temperature at ~30°C. Temperature, salinity and pH were measured daily using a YSI® 63 multiparameter apparatus, whereas dissolved oxygen were measured weekly, using a YSI® 55 oxygen meter. Total ammonia-nitrogen and nitrite-nitrogen concentrations were determined daily according

to SOLORZANO (1969) and STRICKLAND and PARSONS (1972), respectively, using a Hach DR 2000 spectrophotometer (Hach Co., Loveland, Colorado, USA). The water parameters were similar in all treatments and match the commendation for hatchery of *M. amazonicum*, according to MORAES-VALENTI and VALENTI (2010). During the culture, mean temperature was 29.0 ± 1.5°C, salinity was 10 ± 0.5, pH and dissolved oxygen were kept at 8.0 ± 0.5 and 6.5 ± 1.0 mg L^{-1}, respectively. Total ammonia-N and nitrite-N were kept below 0.02 mg L^{-1}, which is much lower than the limits commended by MORAES-VALENTI and VALENTI (2010) and HAYD *et al.* (2014), for the larviculture of *M. amazonicum* (1.6 and 0.8 mg L^{-1}, respectively). The photoperiod was 12 h/12 h (light/dark). In addition to indirect natural light through the windows, cool-white fluorescent lamps were used in the roof.

Experimental Design

It is well known that feed management in hatchery of *Macrobrachium* species should combine *Artemia* nauplii with inert diet because nauplii do not provide the complete nutrient requirements (BARROS and VALENTI, 2003; NAIR *et al*., 2007; SANTOS *et al.*, 2007; DHONT *et al*., 2010). Post-larvae of *M. amazonicum* have been produced with success using a feeding management based on supplying *Artemia* nauplii during all culture and the addition of inert diet from the larval stage V on (MORAES-VALENTI and VALENTI, 2010). ARAÚJO and VALENTI (2007) demonstrated that more than 50% of *M. amazonicum* larvae ingested inert diet from stage V onwards, and that they showed a clear preference for this food from stage VII on. Thus, in the present study, we selected these two stages to start the total replacement of *Artemia* nauplii (AN) by inert diet and use the usual feed management as control. An experiment followed a randomized-complete-blocks design with three feed managements (treatments) and five replicates were performed. Tanks of distinct blocks were stoked in different days. The treatments were: AN-IX – larvae were fed *Artemia* nauplii during all culture and when they reached stage V, inert diet was added to the feed (control); AN-VII – larvae were fed *Artemia* nauplii from stage II to stage VI and it was totally replaced by inert diet from stage VII onwards, whereas both *Artemia* nauplii and inert diet were supplied to stages V and VI; AN-V – larvae were fed *Artemia* nauplii from stage II to stage IV and it was totally replaced by inert diet from stage V onwards.

Feeding Management

The larvae were fed on newly-hatched *Artemia franciscana* nauplii (INVE) and inert diet traditionally called egg-custard. The inert diet (Table 1) was formulated according to MALLASEN and VALENTI (1998); it has a jelly-like consistency and was reduced into particles <1 mm before supply to larvae. The palatability of this inert diet has been demonstrated to be appropriate for *M. amazonicum* larvae in previous experiments. Larvae readily consume it from stage V onwards. Bromatological analysis of the inert diet was conducted in the Ingredients and Pollutant Gases Laboratory at UNESP, based on the standards of the AOAC (1995). The non-nitrogen content is calculated by the difference between the total dry matter and the remaining components of the chemical analysis (Table 1).

Table 1. Diet formulation (according MALLASEN and VALENTI, 1998) and proximate composition of the inert diet used in the present work (based on 100% dry matter).

Ingredients	Percentage (%)
Eggs*	33.66
Squid*	9.90
Fish*	9.90
Dry milk	3.96
wheat flour	1.98
Fish oil	0.79
Vitamin premix[1]	0.69
Mineral premix[1]	0.69
Water	37.42
Vitamin C	0.99
Proximate composition	**Dry matter (%)**
Crude protein	50.60
Crude fat	21.30
Nitrogen-free extract (NFE)	19.80
Minerals	8.30
Dry matter	18.00

** Fresh matter*

[1]AgromixAC50 - basic composition (amount per kg the product): vitamin (vit.) A 176,000 UI, vit. D3 40,000 UI, vit. E 500 mg, vit. K3 120 mg, vit. B1 36 mg, vit. B2 200 mg, vit. B6 70 mg, vit. B12 700 mcg, niacin 750 mg, biotin 3 mg, pantothenic acid 600 mg, folic acid 30 mg, choline 20 mg, iron 1,100 mg, copper 300 mg, manganese 1,800 mg, zinc 1200 mg, iodine 24 mg, selenium 4 mg, methionine 32 g, calcium 180 mg, phosphorus 66 mg, sodium 23 mg, chloride 36 mg, antifungi 200 mg and BHT 1 g.

Artemia nauplii (AN) were supplied once per day, in the early morning, from the second day onwards. The amount was increased according to the larval stage from 4 to 12 AN mL^{-1}, based on MACIEL and VALENTI (2012; Table 2).

The inert diet was supplied twice, at 08:00 h and 12:00 h, but when it was the single feed (in treatments AN-VII and AN-V), the amount was split in five meals during the day at 08:00, 11:00, 14:00, 17:00, and 20:00 h. The mass was increased according to consumption (Table 2). When it wasn't observed leftover feed at the bottom of the tanks at night, feed was increased in the next day.

Table 2. *Artemia* nauplii (AN) and inert diet supplied to larvae in each treatment (Based on MACIEL and VALENTI, 2012).

Day	Dominant stage	*Artemia* nauplii (AN per mL)			Inert diet (g per day)		
		AN -IX	AN -VII	AN -V	AN -IX	AN -VII	AN -V
2	II	4	4	4	-	-	-
3	II-III	6	6	6	-	-	-
4	III	6	6	6	-	-	-
5	III-IV	8	8	8	-	-	-
6-7	IV-V	8	8	8	4	4	10
8	V-VI	10	10	-	5	5	15
9-10	VI-VII	12	12	-	6	6	20
11-12	VII	12	-	-	6	20	25
13-14	VII-VIII	12	-	-	8	30	30
15-16	VII-VIII-IX	12	-	-	8	40	40
17-18	VIII-IX	12	-	-	9	35	35

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

A sample of 10 larvae was removed from each tank every two days and analyzed under an inverted microscope (Olympus® CKX41). It was observed the larval stage based on GUEST (1979), and the gut fullness, gut lipid content, pigmentation, body coloration, setation, muscle to gut ratio, muscle appearance, melanization, fouling organisms and swimming behavior, according to TAYAMEN and BROWN (1999). For each characteristic, larvae was scored as poor (P = 0), satisfactory (P = 1) or excellent (P = 2). Then, larvae were carefully returned to their respective tanks. Based on these data, it was calculated the Larval Stage Index (LSI) and Larval Condition Index (LCI), as follow:

$$LSI = (\sum Si \times ni)/N,$$

in which Si = larval stage (I = 1-9), ni = number of larvae in stage Si, and N = number of larvae analyzed (MANZI *et al.* 1977). LSI varies from 1 to 9.

$$LCI = (\sum Pij)/(10\ N),$$

in which Pij is the score of the larvae i (I = 1-10) in relation to the characteristic j (j = 1-10), and N = total number of larvae analyzed (TAYAMEN and BROWN 1999). LCI varies from 0 to 2.

Fifty larvae were analyzed for each treatment in each sample-day (10 by tank). Post-larvae were not considered for the calculation of the LSI or LCI.

Survival, Productivity and Dry Mass

The culture period ended when more than 80% of the larvae in one of the treatments was metamorphosed into post-larvae. Then, all larvae and post-larvae were harvested and counted. Thus, the survival (larvae + post-larvae), productivity (post-larvae L^{-1}), percent of post-larvae and larvae and mean dry mass (µg) of the post-larvae were calculated. To obtain the dry mass, five samples of post-larvae were taken from each tank and placed in aluminum paper sachets of a known weight. These sachets were then placed on Petri dishes and dried in a stove for 48 h at a temperature of 70°C. The sachets were then transferred to a desiccator for two hours

before being weighed on a balance with an accuracy of 1 μg (Mettler-Toledo Incorporated, ImLangacher, Greifensee, Switzerland).

Data Analysis

For statistical analysis, the data were first tested for normality (Shapiro-Wilk) and homoscedasticity (Brown-Forsythe). As no significant deviation was observed, means were subjected to two-way ANOVA (*F*-test), followed by comparisons between pairs of means using the Tukey post-hoc test (ZAR, 1984). The survival data were square-root arc-sine transformed prior to analysis, but they are presented as non-transformed percentages for easier interpretation. Significance level was set at $P<0.05$. The data were analyzed using the SAS package (version 8.0).

RESULTS

It was observed that larvae of *M. amazonicum* ingested both *Artemia* nauplii (AN) and egg custard readily they were supplied. The consumption of AN by the *M. amazonicum* larvae increased progressively during larvae development up to stage VII, and after that it remain constant in treatment AN-IX, despite of the growth of larvae. On the other hand, the consumption of inert diet increased until stage IX. In addition, it was observed that the digestive tract of larvae was generally full of inert diet from larval stage V onward. When inert diet was the single food available (stages V to IX in AN-V and stages VII to IX in AN-VII), 90-100% of larvae showed digestive tract full of inert diet. In treatment AN-VII, 50% of larvae in stage V and 60-70% of larvae in stage VI showed both AN and inert diet in digestive tract. The first post-larvae appeared on the 12th day of culture, except in the AN-V treatment, where they appeared only after the 15th day. The culture ended on the 19th day after stocking in all treatments, in all blocks.

There were no significant interaction between blocks and treatments and no differences among the blocks ($P>0.05$) for all variables analyzed. The LSI showed significant lower values in the AN-V treatment (Table 3) from the 12th day of the culture period onwards and in the AN-VII in the 16th day of the culture. A reduction in the LCI was observed just following the replacement of AN by inert diet (Figure 1) in both treatments AN-VII and AN-V. In these cases, the reduction in hepatopancreas reserves and the pigmentation of the larvae were the principal factors affecting the LCI, although the digestive tract was generally full in the larvae analyzed. These larvae were generally pale in color, whereas the musculature of the abdomen was opaque and the hepatopancreas was poor in lipids. During the last days of the culture, the atrophy of the abdomen musculature was evident in the animals subjected to AN-V treatment, due to the enlargement of the intestine.

Table 3. Larval Stage Index (LSI) recorded per day of the culture for the larvae of *M. amazonicum* subjected to different feeding regimes. The *Artemia* nauplii (AN) were supplied in AN-V and AN-VII tanks, up to days 7 and 9, respectively. AN-IX – larvae were fed AN during all culture and when they reached stage V, inert diet was added to the feed; AN-VII – larvae were fed AN from stage II to stage VI and it was replaced by inert diet from stage VII onwards; AN-V – larvae were fed AN from stage II to stage IV and it was replaced by inert diet from stage V onwards.

Days	AN -IX	AN-VII	AN- V
2	1.5 ± 0.2 ^{a}	1.7 ± 0.2 ^{a}	1.7 ± 0.3^{a}
4	3.1 ± 0.5 ^{a}	3.0 ± 0.4 ^{a}	2.3 ± 0.3 ^{a}
6	4.7 ± 0.5 ^{a}	4.4 ± 0.2 ^{a}	4.5 ± 0.2 ^{a}
8	5.8 ± 0.8 ^{a}	5.8 ± 0.5 ^{a}	6.0 ± 0.4 ^{a}
10	7.3 ± 0.5 ^{a}	7.3 ± 0.3^{a}	7.0 ± 0.2 ^{a}
12	8.1 ± 0.2^{a}	8.0 ± 0.1 ^{a}	7.4 ± 0.4 ^{b}
14	8.4 ± 0.2 ^{a}	8.4 ± 0.3 ^{a}	7.8 ± 0.3 ^{b}
16	8.6 ± 0.2 ^{a}	8.4 ± 0.2^{b}	8.0 ± 0.1^{b}

Data are expressed as mean ± SD. Different letters on the same line indicate significant ($P<0.05$) differences between the respective means. Fifty larvae were analyzed for each treatment in each sample-day (10 by tank).

The replacement of AN by inert diet resulted in a significant decrease in both survival and productivity (Table 4). This effect was significantly greater in the AN-V treatment in comparison with the AN-VII treatment. The dry mass of the post-larvae was also significantly lower in the AN-V and AN-VII than in AN-XI treatment, in which AN was supplied during all culture (Table 4). Nonetheless, the mean post-larvae mass was not significantly different between the AN-V and AN-VII.

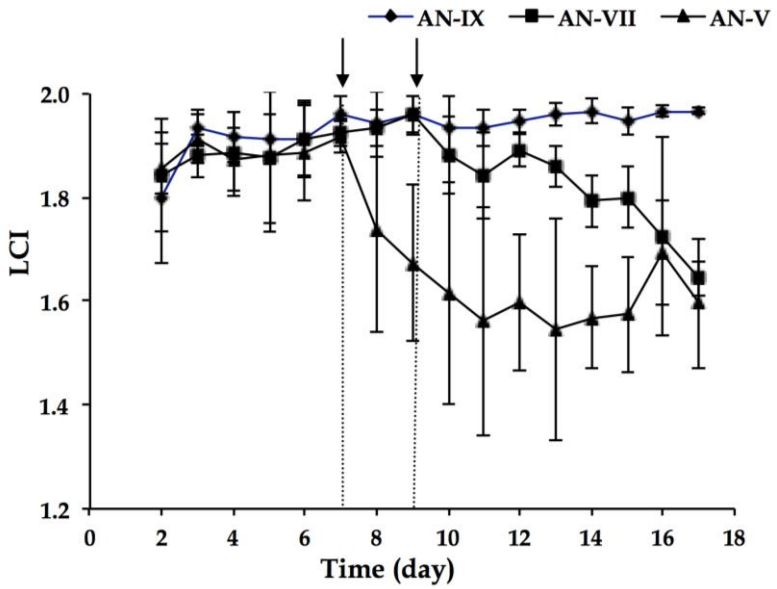

Figure 1. Larval Condition Index (LCI) (mean ± SD) recorded per day of the culture for the larvae of *M. amazonicum* subjected to different feeding regimes. The arrows indicate the days, in which the *Artemia* nauplii (AN) supply was stopped in AN-V and AN-VII. AN-IX – larvae were fed AN during all culture and when they reached stage V, inert diet was added to the feed; AN-VII – larvae were fed AN from stage II to stage VI and it was replaced by inert diet from stage VII onwards; AN-V – larvae were fed AN from stage II to stage IV and it was replaced by inert diet from stage V onwards.

Table 4. Mean (± SD) of survival (%), productivity (PL L^{-1}), post-larvae % and larvae % and dry mass (µg) of the *M. amazonicum* post-larvae submitted to different feeding regimes: AN-IX – *Artemia* nauplii plus inert diet; AN-V – *Artemia* nauplii replaced with inert diet at stage V; AN-VII – *Artemia* nauplii replaced with inert diet at stage VII. (Tukey test, $P<0.05$).

	AN-IX	AN-VII	AN-V
Survival (%)	86.1 ± 6.3^a	56.9 ± 13.7^b	44.0 ± 19.6^b
Productivity (PLL^{-1})	59.7 ± 7.7^a	39.4 ± 8.4^b	9.7 ± 6.5^c
Post-larvae (%)	83.3 ± 11.1^a	85.0 ± 16.3^a	32.4 ± 26.7^b
Larvae (%)	16.7 ± 11.1^a	15.0 ± 16.3^a	67.6 ± 26.7^b
Dry mass (µg)	918 ± 11.0^a	668 ± 12.0^b	698 ± 13.0^b

Different letters on the same line indicate significant differences ($P<0.05$) among the respective means.

DISCUSSION

Feeding larvae of *M. amazonicum* with solely inert diet from stage V or VII onward, negatively affect larval development and survival. The larval time was increased and the general condition was severely affected in most larvae. Survival decreased from ~86% to ~50%, whereas post-larvae (PL) mass declined ~25%. Productivity drastically decreased from ~60 PL L^{-1} to ~40 PL L^{-1} in AN-VII and ~10 PL L^{-1} in AN-V. Larvae largely ingest inert diet, since it was demonstrated by direct observation of the digestive tract. Thus, the differences observed in the present study may be due to the low nutrient quality of inert diet or the low capacity of *M. amazonicum* larvae processing and/or assimilating the nutrients provided by inert diet.

The egg custard used in the present experiment contains the same proportions of proteins and lipids as those described by JONES *et*

al. (1997) for the *Artemia* nauplii (AN). Nevertheless, the profile of amino acids and/or fat acids may be different as well as the content of vitamins and minerals. These differences may be impaired the replacement of AN by the egg custard. On the other hand, it is well known that the nutrient contents of AN is quite variable and the composition fluctuates considerably with strain, harvest season, batch and life stage (DHONT *et al*. 2010). Thus, a diet with stable nutrient profile is not necessary for replacing AN. OHS and D'ABRAMO (1998) tested a diet processed in a spray-dryer based on nutrient composition of zooplankton as an alternative to use of AN in *M. rosenbergii* hatchery. Similarly to the present experiment, larvae survival was low. GARCIA-ORTEGA and HUISMAN (2001) showed that the proteins present in AN are made up of small peptides of low molecular weight and free amino-acids, whereas in artificial diets, generally, a majority of the proteins has a relatively high molecular weight. These authors also point out that assimilation of proteins and their functionality are affected by their interactions with the fatty acids present in the live food. In this case, the structural layout observed in biological systems was not present in the artificial diet. In addition, AN contains carotenoids and astaxantin (GHIDALIA, 1985), which act as co-factors or allosteric components of the molecules involved in enzymatic reactions (LIÑAN-CABELLO *et al*., 2002). NAIR *et al*. (2007) verified that partially replacing AN with a frozen micro-crustacean rich in astaxantin and HUFA, increase production of post-larvae of *M. rosenbergii*. This result suggests the importance of carotenoids for *Macrobrachium* larvae. The above information indicates that, although a fixed nutrient profile is not necessary, a lot of work is needed to provide an inert diet nutritionally suitable for replace AN in larviculture of crustaceans.

The problem for early feeding stages in aquatic organisms may not be the nutritional inadequacy of the diets but rather a lack of assimilation capacity. There are evidences from the present study that the digestibility and assimilation of the nutrients in the inert diet were not effective when ingested alone. Despite the fact that the digestive tracts of the animals were generally full, survival, metamorphosis and mass gain were low in treatments that AN supply stopped at larval stage V and VII. It is well known that AN are deficient to nourish larvae of *M. rosenbergii* (DHONT *et al*. 2010). Previous tests have demonstrated that survival and metamorphosis of *M. amazonicum* larvae is very low when AN are the single feed supplied during all culture (unpublished data). Thus, we can suppose that when larvae fed on AN plus egg custard, a synergic effect has been arisen and improved nutrient assimilation from inert diet. As consequence, it was observed high survival, mass gain and productivity. AN have many proteolytic enzymes (GARCIA-ORTEGA *et al*. 1998). Thus, we can speculate that exogenous enzymes may act in digestive tract of *M. amazonicum* larvae. On the other hand, AN may liberate some stimulants to activate endogen enzymatic mechanisms as discussed above. If so, it would be promising look for the minimum amount of AN to produce such effects rather than completely replace AN. Feeding managements based on low amount of AN supplied could be more cost-effective and efficient. Thus, it should be investigated.

The production of digestive enzymes in *M. amazonicum* larvae during the ontogenetic development is unknown. Nevertheless, low production or reduced specificity is likely during the early larval stages. KAMARUDIN *et al.* (1994) reported that the production of enzymes is low and digestive capacity is restricted in early larval stages of *M. rosenbergii*. Generally, the hepatopancreas becomes more specialized, increase in size and produce more enzymes during development in larvae of decapod crustaceans (JONES *et al*., 1997; AGARD, 1999). In the present study, the significant decrease in survival and metamorphosis rate observed when AN supply is stopped in larval stage V in relation to larval stage VII may reflect similar development in *M. amazonicum*. On the other hand, larvae subjected to treatment AN-VII fed on inert diet together AN from the stage V to VII (2 days), while larvae subjected to AN-V undergone an abrupt interruption. Thus, the supply of the inert diet joint to AN during two days in AN-VII larvae could cause a training feed and/or physiological adaptation for the new food. Nevertheless, no apparent difference in ingestion

of inert feed was observed between larvae of these treatments.

CONCLUSION

In conclusion, the hypothesis that AN may partially be replaced with a practical inert diet (egg custard) to feed larvae of *M. amazonicum* has not been confirmed, although larvae ingest such diet very well. Further research should be conduct to clarify if it is due to unsuitable composition of the egg custard or poor assimilation by larvae. On the other hand, the presence of live food seems to stimulate the efficient assimilation of the inert food. If so, the reduction in the amount of AN supplied together to inert food should be a more cost-effective management.

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