Int Aquat Res
https://doi.org/10.22034/iar.2024.2008912.1731

ORIGINAL RESEARCH

Effect of different crude protein and crude energy levels in diet of broodstock success and larval development of Pantanal freshwater prawn *Macrobrachium pantanalense*

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Received: 06 August 2024 / Accepted: 22 December 2024 / Published online: 02 January 2025

Abstract The goal of this study was to establish the most suitable levels of crude protein and energy in the diet for *Macrobrachium pantanalense* broodstock. The aim was to determine the optimal nutritional requirements and evaluate any potential impact of these diets on reproductive factors, including offspring quality and larval development. Broodstocks were fed six different feeding regimes, each with two levels of crude protein (CP) (30% and 40%) and three levels of crude energy (CE) (3,000, 3,600, and 4,200 kcal/kg). Each regime had five replicates with 6 animals per experimental unit. The resistance of larvae to starvation was evaluated based on the broodstock feeding regimes, and larval development was assessed until metamorphosis to decapodid stage, also with 5 replicates and 6 larvae per experimental unit. The results showed that variable protein and energy crude levels were evaluated in breeders' diet, being found that 30% protein level and 4,200 kcal/kg of energy promoted a higher fertility and larval production. Moreover, larvae resulting from breeders fed on this diet exhibited a mean survival of 8 days when starved, even being able to reach the third zoeal stage under this condition. When cultured to metamorphosis, larvae that exhibited higher survival and a higher metamorphosis synchronism were the ones obtained from breeders fed with a 40% protein level and 3,600 kcal/kg energy crude level (CP:CE) diet. The highest growth values were obtained from whose parental broodstock was fed with a 30% protein level and 4,200 kcal/kg diet.

Keywords Aquaculture . Freshwater prawn . Nutritional requirements . Offspring quality . Reproduction . Wetland

Introduction

Aquaculture production has been growing annually worldwide. In 2022, a total production of 185 million tons was recorded, with crustacean production accounting for approximately 5.16 thousand tons (FAO 2024). In Brazil, the exotic species *Macrobrachium rosenbergii* is the most commonly used freshwater prawn in aquaculture. However, there are native species with economic potential for the activity, such as *Macrobrachium amazonicum* (Moraes-Riodades and Valenti 2001; Moraes-Valenti and Valenti 2010), *Macrobrachium carcinus* and *Macrobrachium acanthurus* (Kutty and Valenti 2010; Valenti 1993) and *Macrobrachium pantanalense* (Dos Santos et al. 2013; Weiss et al. 2015).

The Pantanal freshwater prawn *M. pantanalense*, previously recognized as *M. amazonicum*, was recently described as a new species and can be found in several freshwater ecosystems in the Pantanal of Mato

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Grosso do Sul (Dos Santos et al. 2013; Weiss et al. 2015; Marco-Herrero et al. 2019; Nogueira et al. 2024). This endemic prawn shows great potential for aquaculture (Valenti et al. 2011), the aquarium trade (Karim et al. 2015) and has been studied in several aspects such as biological reproduction (Hayd and Anger 2013; Vercesi and Hayd 2015), nutrition (Freitas et al. 2016, 2021), and as a model species to perform risk assessment trials (Soares et al. 2017, 2019, 2020).

In crustaceans, proteins play a crucial role in body maintenance, growth, reproduction, gonadal maturation, and gamete formation (Romagosa et al. 2012). However, using protein as the primary energy source increases the economic costs of production systems (Silvia et al. 2012). To reduce these costs, it is essential to incorporate primary energy sources into the animal diet. Crude energy is particularly important in prawn reproduction, serving as a fundamental resource for embryogenesis and directly impacting larval quality (Benítez-Mandujano and Ponce-Palafox 2014). Most studies on broodstock nutrition focus on *M. rosenbergii*. While the information from these studies can serve as a basic reference for identifying the nutritional requirements of *M. pantanalense*, experimental validation will be necessary. Cavalli et al. (1999) found that using a diet with 45% of crude protein and 3,900 kcal/kg of energy resulted in improved fecundity and larval production in *M. rosenbergii*. Similarly, Das et al. (1996) reported better results with a diet containing 40% of crude protein and 4,000 kcal/kg of energy. Ribeiro et al. (2012) observed that *M. amazonicum* broodstock exhibited enhanced fecundity with a diet containing 32% of crude protein and 3,660 kcal/kg of energy. These studies demonstrate a range of nutritional levels that can impact reproductive success in crustaceans.

A portion of the energy obtained from the feed is allocated to female nutrition, while the remainder is used for fertilization, maternal growth, and ultimately converted into yolk reserves for newly hatched larvae (Cavalli et al. 1999, 2000). Maternal effects can influence various characteristics of larval production, with potential impacts on offspring performance (Marshall and Uller 2007). The maternal investment ratio can affect embryo quality and larval development, which may be influenced by environmental factors. Katre and Reddy (1982) found that in *Macrobrachium idella*, 82% of the embryo is made up of protein and 11% of lipids. This composition aligns with the energy utilization by larvae, as lipid reduction in body composition is twice as high as protein reduction in the first two developmental stages of *M. rosenbergii* larvae (Roustaian et al. 2001).

Well-fed animals with their nutritional requirements met can efficiently perform basic organism maintenance functions and allocate nutrients towards reproduction (Pezzato et al. 2003). It is crucial to consider the broodstock/offspring ratio and nutrition in this process. Nutrients are transferred from the digestive gland to vitellogenesis, and they must be provided through the diet (Wickins and Lee 2002). Current trials on freshwater prawn nutrition primarily focus on juvenile growth, overlooking the importance of reproduction and the impact of nutrients on quality and larval development. For instance, juveniles *M. pantanalense* exhibit significant growth when fed a diet containing 30% crude protein (Freitas et al. 2016). However, there is a lack of literature on nutritional studies identifying the optimal levels of protein and crude energy in the diet for *M. pantanalense* broodstock (D'Abramo 1998; D'Abramo and New 2010). This paper aims to contribute to filling this gap in knowledge. This study aimed to determine the optimal levels of crude protein and energy in the diet for *Macrobrachium pantanalense* broodstock. The goal was to identify the best nutritional requirements and assess any potential influence of these diets on reproductive aspects, such as offspring quality and larval development.

Materials and methods

All experimental trials were conducted at the Carcinology Laboratory on the Campus of Aquidauana, State University of Mato Grosso do Sul (UEMS), Brazil (20° 28' S, 55° 48' W). Adult *M. pantanalense* were collected from Baiazinha Lake in Miranda, Mato Grosso do Sul, Brazil (20°16" S, 56°23" W). The crustaceans were transported to the laboratory, acclimated, and fed a commercial ration (Alcon Goldfish Colours Bits, purchased from Alcon Pet, SC, Brazil, with a crude protein content of 32% for the first 30 days.

Broodstock trial

Different energy and protein crude levels in the diet of *Macrobrachium pantanalense* broodstock

After an acclimatization period, 48 males and 96 females were randomly selected and stocked in 24 aquariums (25 L) with a life support system containing a biological filter (20% of the total volume), following the method adapted from Calado et al. (2007). Each aquarium housed 6 animals (1:2 sexual ratio). The experiment tested two levels of crude protein (30 and 40%) and three levels of crude energy (3,000, 3,600, and 4,200 kcal/kg) in the broodstock diet.

A completely randomized design was used, consisting of six experimental diets (Table 1) formulated using the Super Crac 5.7 Master software from TD Softwares, Viçosa – Minas Gerais, Brazil. The diets were based on the dry matter of ingredients outlined in Rostagno et al. (2024) and the nutritional requirements for *M. rosenbergii* (D'Abramo and New 2010), *M. amazonicum* (Pezzato et al. 2003) and *M. pantanalense* juveniles (Freitas et al. 2016) (Table 1). The ingredients were homogenized, hydrated for pelletizing, and processed into granules (0.1 mm) before being dried in a forced-air kiln for 72 hours at 55°C, following the method by Pezzato et al. (2003). The chemical composition of the diets was analyzed in the animal nutrition laboratory at the Campus of Aquidauana, State University of Mato Grosso do Sul (UEMS), Brazil, as described by Detmann et al. (2012).

After an initial biometric measurement of the broodstock, the feed proportion was adjusted to 10% of the total biomass. Starting from the second day, the total amount of feed was modified according to the residual amount, with feeding occurring four times a day over a period of 90 days. Ovigerous females were monitored daily using the eggs maturation scale (Hayd and Anger 2013; Vercesi and Hayd 2015), and the larvae were collected, counted and separated after hatching. Water quality parameters, including dissolved oxygen (8 ± 1 mg/L), temperature (28°C ± 2°C), pH (6.5 ± 0.5), and electrical conductivity (0.001 ± 0.01 mS/cm) in the aquariums were monitored daily using a "Yellow Springs Instruments" (YSI 556) multi-parameter probe. Ammonia (NH_4^+) and nitrite (NO_2^-) levels were measured twice a week using Labcon Test colorimetric kits, with both parameters maintained around 0.00.

Evaluation parameters

At the end of the 90-day experiment, the test was completed, and all data collected during this period were

Table 1 Percentage and chemical composition of the experimental diets, containing different crude protein and crude energy levels in the feeding regime of *Macrobrachium pantanalense* broodstock.

Ingredient	Feed regime					
	30 CP 3,000 CE	30 CP 3,600 CE	30 CP 4,200 CE	40 CP 3,000 CE	40 CP 3,600 CE	40 CP 4,200 CE
Fish Meal (54) (%)	0.3500	0.3500	0.3500	0.3500	0.3500	0.3500
Conc. Prot. Soy (60)[1] (%)	0.1600	0.1600	0.1600	0.3200	0.3200	0.3200
Corn meal (%)	0.0605	0.0785	0.0785	0.0605	0.0605	0.0785
Rice husk (%)	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500
Soy Oil (%)	0.0020	0.0240	0.0560	0.0000	0.0125	0.0460
Fish Oil (%)	0.0020	0.0240	0.0560	0.0000	0.0125	0.0460
Inert (%)	0.1595	0.0745	0.0000	0.1850	0.0800	0.0050
Starch (%)	0.1765	0.2000	0.2105	0.0000	0.0800	0.0700
Limestone (%)	0.0210	0.0210	0.0210	0.0225	0.0225	0.0225
Mono. phosphate.[2] (%)	0.0065	0.0060	0.0060	0.0000	0.0000	0.0000
Premix[3] (%)	0.0100	0.0100	0.0100	0.0100	0.0100	0.0100
BHT[4] (%)	0.0020	0.0020	0.0020	0.0020	0.0020	0.0020
Total (kg)	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Nutrient						
Crude protein (%)	29.97	30.12	30.13	39.96	40.01	40.14
Crude energy (kcal/kg)	3,069.10	3,609.20	4,203.10	3,088.60	3,604.30	4,218.50
Ether extract (%)	3.33	7.73	14.03	2.97	5.45	12.11
Crude Fibre (%)	2.57	2.57	2.57	2.99	2.99	3.02
Calcium (%)	3.02	3.02	3.02	3.01	3.01	3.01
Phosphorus (%)	1.50	1.50	1.50	1.72	1.72	1.72

[1] Soybean protein concentrate; [2] Monocalcium phosphate; [3] Premix vitamin and mineral - composition (per kg of product / Migfish 1%): Folic Acid: 299.88 mg, Ascorbic acid: 15,000.12 mg, Pantothenic acid: 3,000.10 mg, biotin: 0.06 mg, Niacin (B3): 9,000.32 mg, choline (B4): 103,500.00 mg, Vit. A: 1,000,000.00 UI, Vit. B1: 1,500.38 mg, Vit. B2: 1,500.0 mg, Vit. B6: 1,500.38 mg, Vit. D3: 240,000.00 UI, Vit. E: 10,000.00 mg, Vit. K3: 400.00 mg, Inositol: 9,999.92 mg, Iron: 6,416.80 mg, Manganese: 8,000.40 mg, Copper: 1,000.00 mg, Zinc: 13,999.50 mg, Iodine: 45.36 mg, Cobalt: 60.06 mg, Selenium: 60.30 mg, Magnesium: 5.10 mg, Chlorine: 2.30 %, Sulphur: 0.01 %; [4] Butyl Hydroxytoluene (BHT).

used to determine the following reproductive parameters: larval production, estimated by the quantity of newly hatched larvae in a fecundation per female; fertility, estimated as the number of newly hatched larvae per female weight (larvae/female weight); reproductive effort (RE), the percentage of effort devoted to reproduction per spawning event (newly hatched larvae weight / female weight x 100); and the weight (mg) of the newly hatched larvae (zoea I). The results were expressed as mean values with standard deviations, as well as the maximum and minimum values recorded (Table 2).

Larvae trial

Larvae starvation resistance from broodstock fed with diets containing different crude protein and energy levels

Twenty newly hatched larvae were used from each of the 6 feeding regimes, with 2 females per treatment. The experiment was conducted in polyethylene 6-well microplates with a minimum of 4 replicates per treatment. Each replicate consisted of 5 larvae in the starvation regime, placed in one well of the microplate with 10 ml of water. Water changes were performed daily, and the larvae were maintained in a chamber with controlled temperature (28°C), salinity (4), and a photoperiod of 12 hours light and 12 hours dark

Table 2 Mean value, standard deviation, maximum and minimum values of larval production, fertility, reproductive effort (RE), newly hatched larval weight (ZI) in *Macrobrachium pantanalense* broodstock fed with diets containing different crude protein and crude energy levels.

Evaluated parameters	30 CP 3,000 CE	30 CP 3,600 CE	30 CP 4,200 CE	40 CP 3,000 CE	40 CP 3,600 CE	40 CP 4,200 CE
Larvae production (max.)	81	127	162	73	133	71
Larvae production (X ± SD)	55 ± 7^{b}	63 ± 7^{b}	104 ± 6^{a}	39 ± 7^{b}	78 ± 7^{ab}	42 ± 9^{b}
Larvae production (min.)	13	29	40	16	50	26
Fertility (max.)	167	169	308	194	229	136
Fertility (X ± SD)	104 ± 11^{b}	121 ± 13^{b}	176 ± 11^{a}	93 ± 13^{b}	135 ± 11^{ab}	87 ± 15^{b}
Fertility (min.)	44	79	69	36	87	60
RE (%) (max.)	88.74	99.78	95.56	100.59	71.43	79.13
RE (%) (X ± SD)	52.59 ± 5.52	62.49 ± 6.15	59.54 ± 6.88	57.67 ± 5.40	69.39 ± 5.87	69.40 ± 5.40
RE (%) (min.)	46.77	32.55	23.95	36.06	30.02	43.38
Weight ZI (mg) (max.)	4.30	3.60	3.40	3.40	3.50	3.50
Weight ZI (mg) (X ± SD)	2.66 ± 0.18	3.04 ± 0.20	2.95 ± 0.23	3.02 ± 0.18	2.99 ± 0.19	2.76 ± 0.17
Weight ZI (mg) (min.)	2.60	2.40	1.60	2.40	2.40	2.70

Mean values fellow by different letters in line presented statistical differences (Tukey P < 0.05). (Acronyms: max – maximum value; min – minimum value; X – Mean value; SD – Standard deviation; ZI – Zoea I)

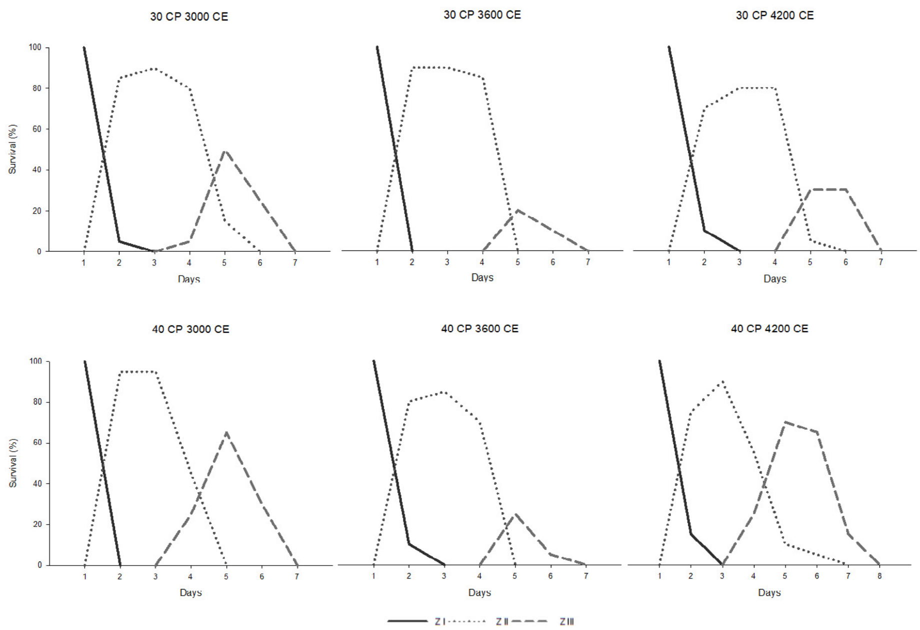

Fig. 1 Survival in days of *Macrobrachium pantanalense* larvae in the starvation regime. Larvae from broodstock fed diets containing different crude protein and crude energy levels. (Z – stage of larvae development)

(TE-401 model, Tecnal, Brazil) (Freitas et al. 2021). Brackish water was prepared by dissolving marine salt (Nutratec) in distilled water.

Evaluation parameters

The larval development was assessed by identifying the developmental stages after each molting stage (Marco-Herrero et al. 2019) using a digital magnifying glass (Opticam HD 3.7) until death. The results were expressed as the percentage of larvae at each developmental stage per day (Figure 1).

Larval development of *Macrobrachium pantanalense* from broodstock developed under different feeding regimes

Thirty newly hatched larvae from each feeding regime, obtained from a pool of 2 females per treatment, were individually cultured in 100 ml glass containers until metamorphosis. The larvae were maintained at a salinity of 4, a temperature of approximately 28°C, and a photoperiod of 12:12h (light:dark). They were fed daily with newly hatched Artemia nauplii (8 nauplii/ml) following the method adapted from Maciel et al. (2012). After metamorphosis, the weight and length of the decapodid were measured. Total length (TL) was measured from the tip of the rostrum to the tip of the telson, and carapace length (CL) was measured from the distal tip of the rostrum to the posterior extremity of the cephalothorax using a digital magnifying glass (Opticam HD 3.7). A linear regression equation was derived from these measurements: $TL = 3.4215\ CL + 2.0269$, $R^2 = 0.99$. For weight determination, the larvae were freeze-dried (Enterprise I – Terroni) for 24 hours and weighed using an analytical balance (Ohaus Adventure).

Evaluation parameters

The larval development was assessed by identifying the developmental stages after each molting stage, allowing for the evaluation of development, survival, and percentage of decapodid in each treatment. This percentage was calculated as the ratio of larval amount to the developmental stage on the observed day. The synchronization of metamorphosis to decapodid in days post-hatch was also evaluated. The number of larvae that completed metamorphosis from the first decapodid to the last in days was recorded. These values were then transformed and presented as a percentage to analyse the metamorphosis trend. Results for larval growth, including total length and weight, are presented as mean values with standard deviations, as well as the maximum and minimum recorded values (Table 3).

Statistical analysis

The reproductive aspects, including larval production, fertility, reproductive effort, and zoea weight, as well as larval growth in terms of total length and weight, were analyzed using a two-way ANOVA to examine the effects of protein and energy levels on these variables (Rohlf and Sokal 1995). After verifying the assumptions of normality and homogeneity of variance through the Kolmogorov-Smirnov and Levene tests, respectively, statistical analysis was conducted. When significance was observed at $P < 0.05$, Tukey's multiple comparison tests were employed to compare the means. The analysis was carried out using SigmaPlot 13.0 software.

Table 3 Mean value, standard deviation, maximum and minimum values of total length (TL) and weight of *Macrobrachium pantanalense* larvae from broodstock fed diets containing different crude protein and crude energy levels.

Evaluated parameters	30 CP 3,000 CE	30 CP 3,600 CE	30 CP 4,200 CE	40 CP 3,000 CE	40 CP 3,600 CE	40 CP 4,200 CE
TL (mm) (max.)	8.80	7.12	7.26	7.77	7.53	7.22
TL (mm) (X ± SD)	6.87 ± 0.09	6.66 ± 0.11	6.86 ± 0.11	6.58 ± 0.10	6.76 ± 0.10	6.65 ± 0.11
TL (mm) (min.)	6.03	6.23	5.64	5.55	6.16	5.92
Weight (mg) (max.)	4.52	4.30	4.00	4.00	4.40	4.30
Weight (mg) (X ± SD)	2.71 ± 0.15	2.86 ± 0.17	3.07 ± 0.17	2.97 ± 0.15	2.76 ± 0.15	2.60 ± 0.17
Weight (mg) (min.)	1.25	1.90	1.90	1.70	1.80	1.40

Acronyms: Max – maximum value; min – minimum value; X – Mean value; SD – Standard deviation ($P > 0.05$

Results

Different crude protein and energy levels in the diet of broodstock

There was an interaction between the crude protein and crude energy levels for larval production and fertility (P < 0.05) while there was no interaction for reproductive effort and zoea I weight (P > 0.05). Larval production was higher in the diet 30 CP/4,200 CE, with 104 ± 6 larvae, being statistically equal to feed regime 40 CP/ 3,600 CE, with 78 ± 7 larvae (P > 0.05) however, the feed regime 30 CP/ 4,200 CE was statistically different of the others feed regimes (P > 0.05) (Table 2). The fertility of the feed regime 30 CP/ 4,200 CE, with 176 ± 11, were higher than others diets (P < 0.05) being similar only to feed regime 40 CP/ 3,600 CE, with 135 ± 11 (P > 0.05). The highest production and fertility values of larvae were recorded in the feed regime 30 CP / 4,200 CE (P < 0.05). The reproductive effort was lower in the followed feed regimes: 30 CP/3,000 CE (52.59 ± 5.52%); 40 CP /3,000 CE (57.67 ± 5.40%); and 30 CP/4,200 CE (59.54 ± 6.88%), respectively, there were no significant differences (P > 0.05) observed. The feed regime of 30 CP/ 3,600 CE presented the highest weight larvae (3.04 ± 0.20 mg), and the feed regime 30 CP/ 3,000 CE showed the lowest weight larvae (2.66 ± 0.18 mg). However, the feed regimes were not statistically different (P > 0.05).

Larvae starvation resistance from broodstock fed with different diets

In the larvae development under starvation, all the feeding regime diets achieved the third stage of larvae development. During larval development under starvation, all feeding regimes enabled larvae to reach the third stage. Most larvae survived for 7 days in all treatments, except for the 40 CP/4,200 CE diet, where some survived up to 8 days, at the end of the experiment. There were no significant differences in molting period among the feed regimes (P > 0.05). The 40 CP/4,200 CE diet had the highest survival rates towards the end of the trial (Figure 1).

Larval development from broodstock developed under different feeding regimes

There was no significant interaction between the protein and energy factors on the total length and weight of the decapodid (P > 0.05). The highest total length was observed in the 30 CP/3,000 CE feeding regime, measuring 6.87 ± 0.09 mm, while the lowest total length was seen in the 40 CP/3,000 CE regime at 6.58 ± 0.10 mm (P > 0.05) (Table 3). The highest weight was recorded in the 30 CP/4,200 CE regime, with 3.07 ±

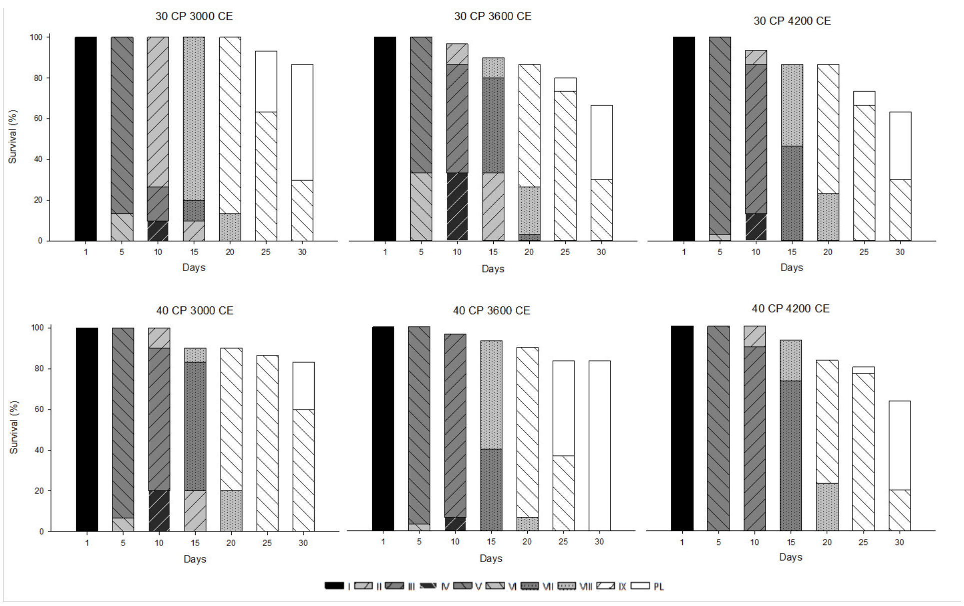

Fig. 2 Survival and larval development in days of *Macrobrachium pantanalense* larvae from broodstock fed diets containing different crude protein and crude energy levels. (PL – post-larvae stage)

0.17 mg, while the lowest weight was in the 40 CP/4,200 CE regime at 2.60 ± 0.17 mg (P > 0.05). Larval development did not differ significantly between the feeding regimes (P > 0.05), but survival rates were above 80% at the end of 30 days in the 30 CP/3,000 CE, 40 CP/3,000 CE, and 40 CP/3,600 CE feeding regimes (Figure 2).

All diets resulted in larval survival rates above 60%, with the highest percentage of decapodid observed in the 40 CP/3,600 CE diet. Some diet treatments showed early settlement as early as 21 days post-hatch, but by 28 days post-hatch, over 50% of larvae in all feeding regimes had settled and were undergoing metamorphosis. Only the 40 CP/3,600 CE regime had 100% of surviving larvae metamorphose by the end of 30 days. The majority of settlements occurred between 25 and 27 days post-hatch (Figure 3).

Discussion

Different crude protein and energy levels in the diet of broodstock

There is a lack of detailed studies on *M. pantanalense* broodstock, their nutritional requirements at different life stages, and feeding habits (Portz and Furuya 2012; Freitas et al. 2021;Ballester et al. 2023). Therefore, feed formulations for this species are based on estimates from studies on *M. rosenbergii* or *M. amazonicum*. Increasing energy in the diet through lipid sources like oils can save energy from protein and optimize protein utilization for growth (Benítez-Mandujano and Ponce-Palafox 2014; Méndez-Martínez et al. 2018), benefiting embryogenesis. The high larval production and fertility of *M. pantanalense* broodstock may be linked to feed ingredients, particularly animal and vegetable oils. Soy and fish oils are crucial for providing energy for embryogenesis and essential fatty acids (Garcia et al. 2012). Freshwater species, like *M. pan-*

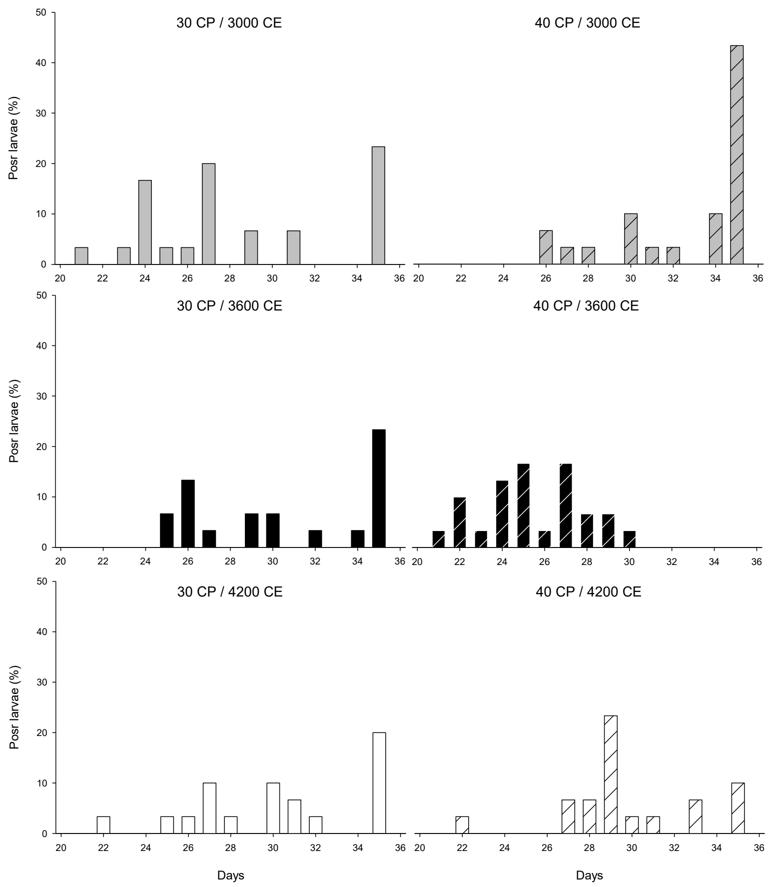

Fig. 3 Metamorphosis synchronization of *Macrobrachium pantanalense* larvae to decapodid in days post-hatch from the first metamorphosis. Larvae from broodstock-fed diets containing different crude protein and crude energy levels.

tanalense, have a high requirement for linoleic acid (LA) n-6, which can be supplied by soy oil or fish oil from species like tilapia (*Oreochromis niloticus*) (Silvia et al. 2012). Studies have shown that higher LA levels in the diet of *M. amazonicum* broodstock lead to increased egg production and fertility, similar to findings in *M. rosenbergii* (Das et al. 1996; Ribeiro et al. 2012). Caridean prawns can convert saturated fatty acids in the diet into monounsaturated forms, storing them in the digestive gland and transporting them to reproductive glands and oocytes (Cavalli et al. 2000; Ribeiro et al. 2012).

The study observed reproductive effort averages ranging from 52% to 70%, which were higher than the results reported by Cavalli et al. (1999) for *M. rosenbergii* broodstock, which showed a reproductive effort between 11% and 13%. The weights of newly hatched larvae were similar to those observed for *M. amazonicum* and *M. rosenbergii* larvae in the initial stage of larval development (Cavalli et al. 1999; Anger & Hayd 2010). The similarity in newly hatched larval weight across all feeding regimes may be attributed to species-specific factors. It is important to note that protein is not a limiting factor that directly affects reproduction and larval quality. Crustaceans primarily utilize energy for embryogenesis, and a diet with lower crude protein content (30%) can be more effective in minimizing the economic costs associated with protein acquisition and use (El-Sayed and Kawanna 2008; Hu et al. 2008). Furthermore, there is potential to optimize practical diets for both laboratory experiments and commercial aquaculture by considering the higher value of crude protein in diet formulations (Cortes-Jacinto et al. 2003; Silvia et al. 2012; Mantoan et al. 2021).

The knowledge of the actual crude protein levels in the broodstock diet is limited for prawns of the *Macrobrachium* genus, ranging from 30% to 45% for species such as *M. amazonicum*, *M. rosenbergii*, and *M. carcinus* (Das et al. 1996; Cavalli et al. 1999; Cavalli et al. 2000; Ribeiro et al. 2012; Benítez-Mandujano and Ponce-Palafox 2014). During the reproductive stage, protein intake by freshwater prawns from their diet is crucial for somatic growth and potentially for reproductive investment as an energy source. It is essential to determine and adjust the energy and protein levels, as well as other nutrients, to meet the specific requirements of different species within this genus, for both males and females, as they play a vital role in the quality of the offspring produced (Romagosa et al. 2012; Rodríguez-González et al. 2014; Craig et al. 2017). The weight of newly hatched larvae may not be directly linked to maternal nutrition, but it could be a species-specific trait to produce offspring of similar size. It is possible that females can allocate additional energy and protein from their diet to their offspring, although there is a lack of previous studies showing a correlation between protein levels in the diet and increased fertility or larvae quality (Racotta 2003).

Larvae starvation resistance from broodstock fed with different diets

The larvae of *M. amazonicum* and *M. pantanalense* (previously recognized as *M. amazonicum*) exhibit obligatory lecithotrophy in the first stage (zoea I) relying solely on their yolk reserve without consuming external sources. In the second stage (zoea II), they display facultative lecithotrophy, being able to consume external food sources and metabolize their yolk reserves for nourishment (Anger and Hayd 2009, 2010). *M. amazonicum* shows greater resistance to starvation, surviving up to 15 days, while *M. pantanalense* has lower resistance, surviving only up to 9 days (Anger and Hayd 2010). The interaction of factors such as crude protein and energy was found to be independent and not decisive in larval development. This complexity suggests that these factors may have more significant impact on reproductive parameters rather than on larval quality and development.

Protein plays a crucial role in the development of larvae, as it is the main component of the organism, representing nearly 60% of the body composition of *M. rosenbergii* larvae. Protein levels increase as larvae develop (Roustaian et al. 2001; Mantoan et al. 2021). Unfed *M. rosenbergii* larvae experience a decrease in lipid levels, likely due to lipid breakdown, which helps conserve body protein in zoea II. The composition of *M. rosenbergii* larvae includes high levels of glutamic acid and phenylalanine + cystine (Roustaian et al. 2000). This information is valuable for formulating diets for broodstock, as amino acid supplementation can directly impact offspring quality and larval development. Larval development is characterized by an increase in protein levels and a decrease in lipid levels. Lipids make up approximately 34% of larval content, but this percentage decreases significantly as yolk reserves are consumed, particularly in the early development stages (zoea I and II) (Roustaian et al. 2001; Kamarudin and Roustaian, 2002). The breakdown of lipids allows the energy from maternal sources to be converted into metabolic energy for tissue synthesis, development, and larval growth (Anger and Hayd 2009; Augusto et al. 2020).

Larval development from broodstock developed under different feeding regimes

The larvae showed superior growth performance in the feed regime with 30 CP/ 4,200 CE digestible energy, possibly due to increased energy in the broodstock diet, leading to higher yolk reserves in the eggs. Katre and Reddy (1982) observed changes in the chemical composition of *Macrobrachium lamarrei* eggs, with an increase in fat content and a decrease in protein. This selective suppression of lipids allows for their catabolism during the early larval stages in scenarios of obligatory and facultative primary lecithotrophy. Larvae of *Lysmata seticaudata* that settle earlier but are smaller in size may develop better in subsequent life stages than larvae that delay settlement and are larger (Carvalho and Calado 2018). The feed regime 30 CP/ 4,200 CE digestible energy feed regime showed similar results in settlement synchronization with a delay of approximately 2 days, indicating its suitability for commercial applications (Martinez-Cordova et al. 2003; Silvia et al. 2012). Postlarvae larger than smaller postlarvae intraspecifically tend to cannibalize each other and prevent smaller postlarvae from accessing food. Size differences in postlarvae can lead to intraspecific competition, resulting in heterogeneous growth and smaller, poorer postlarvae. Standardized larval culture protocols are necessary to reduce group heterogeneity, improve post-settlement growth performance, and ensure high synchrony at metamorphosis to reduce intraspecific cannibalism (Romano and Zeng 2017).

Conclusions

This study confirmed that Pantanal freshwater prawn, *Macrobrachium pantanalense* broodstock should be fed a diet containing 30% crude protein and 4,200 kcal/kg of crude energy. This protein requirement for reproduction is lower than what is commonly used for prawns of this genus. Additionally, a higher level of crude energy was found to be crucial for embryogenesis, fertility, and larval quality, and can be provided through the diet. Further research is needed to better understand the nutritional needs of Pantanal freshwater prawn *M. pantanalense* and to identify appropriate ingredients to enhance the quality of yolk reserves in newly hatched larvae and promote optimal larval development. These findings highlight the potential benefits of low protein requirements in the diet and efficient assimilation of high crude energy in animal production.

Funding This work was supported by the Foundation of Support to Development, Education, Science, and Technology (FUNDECT) under Grant 23/200.755/2014.

Credit authorship contribution statement E.F., K.V., and M.S., performed the research, designed the study, analyzed the data and wrote the manuscript. LH, designed the study, analyzed the data and wrote and reviewed the manuscript and provided funding.

Competing interests The authors declare that they have no competing interests.

Acknowledgments We would like to thank Dr. Ricardo Calado (University of Aveiro) for the comments in this article.

References

Anger K, Hayd L (2010) Feeding and growth in early larval shrimp *Macrobrachium amazonicum* from the Pantanal, southwestern Brazil. Aquat Biol 9:251–261; doi:10.3354/ab00259

Anger K, Hayd L (2009) From lecithotrophy to planktotrophy: ontogeny of larval feeding in the Amazon River prawn *Macrobrachium amazonicum*. Aquat Biol 7:19–30

Augusto A, New MB, Santos MR, Amorim RV, Valenti WC (2020) Energy budget and physiology in early ontogenetic stages of the Amazon river prawn. Aquac Reports 18:100446; doi:10.1016/j.aqrep.2020.100446

Ballester ELC, Dos Santos LD, Piovesan V, Zadinelo IV (2023) Gross energy levels in practical diets for the Amazon river prawn postlarvae, *Macrobrachium amazonicum*. Lat Am J Aquat Res 51:324–330; doi:10.3856/vol51-issue2-fulltext-2965

Benítez-Mandujano M, Ponce-Palafox JT (2014) Effects of different dietary of protein and lipid levels on the growth of freshwater prawns (*Macrobrachium carcinus*) broodstock. Rev MVZ Cordoba 19:3921–3929; doi:10.21897/rmvz.112

Calado R, Vitorino A, Dionísio G, Dinis MT (2007) A recirculated maturation system for marine ornamental decapods. Aquaculture 263:68–74; doi:10.1016/j.aquaculture.2006.10.013

Carvalho L, Calado R (2018) Trade-offs between timing of metamorphosis and grow-out performance of a marine caridean shrimp juveniles and its relevance for aquaculture. Aquaculture 492:97–102; doi:10.1016/j.aquaculture.2018.04.007

Cavalli RO, Lavens P, Sorgeloos P (1999) Performance of *Macrobrachium rosenbergii* broodstock fed diets with different fatty acid composition. Aquaculture 179:387–402; doi:10.1016/S0044-8486(99)00173-8

Cavalli RO, Menschaert G, Lavens P, Sorgeloos P (2000) Maturation performance, offspring quality and lipidcomposition of *Macrobrachium rosenbergii* females fed increasing levels of dietary phospholipids. Aquac Int 8:41–58; doi:10.1023/A:1009220932268

Cortes-Jacinto E, Villarreal-Colmenares H, Civera-Cerecedo R, Martinez-Cordova R (2003) Effect of dietary protein level on growth and survival of juvenile freshwater crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). Aquac Nutr 9:207–213; doi:10.1046/j.1365-2095.2003.00241.x

Craig SR, Gardner TR, Carnevali O (2017) Growout and broodstock nutrition. In: Marine ornamental species: *Aquaculture*. Wiley-Blackwell. 139–158

D'Abramo LR (1998) Nutritional requirements of the freshwater prawn *Macrobrachium rosenbergii*: Comparisons with species of penaeid shrimp. Rev Fish Sci 6:153–163; doi:10.1080/10641269891314267

D'Abramo LR, New MB (2010) Nutrition, feeds and feeding. In: *Freshwater prawns:* Biology and farming (New MB, Valenti WC, Tidwell JH, D'Abramo LR, Kutty MN). Wiley – Blackwell:EUA. 570

Das NN, Saad CR, Ang KJ, Law AT, Harmin SA (1996) Diet formulation for *Macrobrachium rosenbergii* (de Man) broodstock based on essential amino acid profile of its eggs. Aquaculture 27:543–555; doi:10.1111/j.1365-2109.1996.tb01285.x

Detmann EMAS, Valadares Filho SC, Queiroz AC, Berchiellin TT, Saliba EOS (2012) *Métodos para análise de alimentos.* 1 edição. Editora Suprema:Visconde do Rio Branco, MG

Dos Santos A, Hayd L, Anger K (2013) A new species of *Macrobrachium* Spence Bate, 1868 (Decapoda, Palaemonidae), *M. pantanalense*, from the Pantanal, Brazil. Zootaxa 3700:534–546; doi:10.11646/zootaxa.3700.4.2

El-Sayed AFM, Kawanna M (2008) Effects of dietary protein and energy levels on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock in a recycling system. Aquaculture 280:179–184; doi:10.1016/j.aquaculture.2008.04.030

FAO (2024) The state of world fisheries and aquaculture (2024) State World Fish Aquac 2024; doi:10.4060/CD0683EN

Freitas E, Hayd L, Calado R (2021) Effects of salinity, stocking density and feeding in *Macrobrachium pantanalense* larviculture. Aquac Reports 20:100706; doi:10.1016/j.aqrep.2021.100706

Freitas E, Passos F, Hayd L (2016) Diferentes níveis proteicos no crescimento de juvenis de *Macrobrachium pantanalense*. Arch Zootec 65:43–49

Garcia AS, Gonçalves LU, Cavalli, RO Viegas EMM (2012) Lipídeos. In: Nutriaqua: nutrição e alimentação de espécies de interesse para aquicultura brasileira. (Fracalossi DM, Cyrino JEP). Sociedade Brasileira de Aquicultura e Biologia Aquática:Florianópolis, SC. 79–99.

Hayd L, Anger K (2013) Reproductive and morphometric traits of *Macrobrachium amazonicum* (Decapoda: Palaemonidae) from the Pantanal, Brazil, suggests initial speciation. Rev Biol Trop 63:39–57

Hu Y, Tan B, Mai K, Ai Q, Zheng S, Cheng K (2008) Growth and body composition of juvenile white shrimp, *Litopenaeus vannamei*, fed different ratios of dietary protein to energy. Aquac Nutr 14:499–506; doi:10.1111/j.1365-2095.2007.00555.x

Kamarudin MS, Roustaian P (2002) Growth and fatty acid composition of freshwater prawn, *Macrobrachium rosenbergii*, larvae fed diets containing various ratios of cod liver oil-corn oil mixture. J Appl Ichthyol 18:148–153; doi:10.1046/j.1439-0426.2002.00331.x

Karim HM, Freitas JEC, Lima TPC, Nascimento MS, Hayd L (2015) Viabilidade econômica da produção do camarão-do-pantanal (*Macrobrachium pantanalense*). Bol do Inst Pesca 41:103–112

Katre S, Reddy SR (1982) Crustacean egg size as an indicator of egg fat/protein reserves. Int J Invertebr Reprod 4:381–384; doi:10.1080/01651269.1982.10553447

Kutty MN, Valenti WC (2010) Culture of other freshwater prawn species. In: Freshwater prawns: *Biology and farming* (New MB, Valenti WC, Tidwell JH, D'Abramo LR, Kutty MN). Wiley – Blackwell:EUA. 570

Maciel CR, New MB, Valenti WC (2012) The predation of Artemia nauplii by the larvae of the Amazon river prawn , *Macrobrachium amazonicum* (Heller 1862), is affected by prey density, time of day, and ontogenetic development. J World Aquac Soc 43:659–669

Mantoan P, Ballester E, Ramaglia AC, Augusto A (2021) Diet containing 35% crude protein improves energy balance, growth, and feed conversion in the Amazon river prawn, *Macrobrachium amazonicum*. Aquac Reports 21:100962; doi:10.1016/j.aqrep.2021.100962

Marco-Herrero E, Anger K, Hayd L (2019) Extended larval development in the hololimnetic shrimp *Macrobrachium pantanalense* (Decapoda, Palaemonidae) reared in the laboratory. Crustaceana 92:693–723; doi:10.1163/15685403-00003899

Marshall DJ, Uller T (2007) When is a maternal effect adaptive? Oikos 116:1957–1963; doi:10.1111/j.2007.0030-1299.16203.x

Martinez-Cordova LR, Campana Torres A, Porchas-Cornejo MA (2003) Dietary protein level and natural food management in the culture of blue (*Litopenaeus stylirostris*) and white shrimp (*Litopenaeus vannamei*) in microcosms. Aquac Nutr 9:155–160; doi:10.1046/j.1365-2095.2003.00235.x

Méndez-Martínez Y, García-Guerrero MU, Arcos-Ortega FG, Martínez-Córdova LR, Yamasaki-Granados S, Pérez-Rodríguez JC (2018) Effect of different ratios of dietary protein-energy on growth, body proximal composition, digestive enzyme activity, and hepatopancreas histology in *Macrobrachium americanum* (Bate 1868) prawn juveniles. Aquaculture 485:1–11; doi:10.1016/J.AQUACULTURE.2017.11.012

Moraes-Riodades PM, Valenti WC (2001) Freshwater prawn farming in brazilian Amazonia shows potential for economic, social development. The Advocate 1:73–74

Moraes-Valenti P, Valenti WC (2010) Culture of the Amazon river prawn *Macrobrachium amazonicum*. In: Freshwater prawns: *biology and farming* (New MB, Valenti WC, Tidwell JH) Wiley – Blackwell:EUA. 485–497

Nogueira CS, Costa RC, Pantaleão JAF (2024) Variation in larval traits between closely related species of freshwater prawns (*Macrobrachium amazonicum* and *M. pantanalense*). Acta Zool 105:524–538

Pezzato LE, Barros MM, Sampaio FG, Falcon R, Sampaio G, Hisano H (2003) Relação energia: proteína dietária para pós-larvas de *Macrobrachium amazonicum* (Crustacea, Decapoda). Acta Sci 25:235–241

Portz L, Furuya WM (2012) Energia, proteína e aminoácidos. In: Nutriaqua: nutrição e alimentação de espécies de interesse para aquicultura brasileira. (Fracalossi DM, Cyrino JEP). Sociedade Brasileira de Aquicultura e Biologia Aquática:Florianópolis, SC. 65–77

Racotta I (2003) Shrimp larval quality in relation to broodstock condition. Aquaculture 227:107–130; doi:10.1016/S0044-

8486(03)00498-8
Ribeiro K, Franceschini-Vicentini IB, Papa LP, New MB, Valenti WC (2012) Effect of polyunsaturated fatty acids on the fecundity of the Amazon river prawn *Macrobrachium amazonicum* (Heller, 1862). Aquac Res 43:1756–1763; doi:10.1111/j.1365-2109.2011.02980.x
Rodríguez-González H, Hernández-Llamas A, García-Ulloa M, Racotta IS, Montoya-Mejía M, Villarreal H (2014) Effect of protein and lipid levels in diets for female red claw crayfish *Cherax quadricarinatus* on quality of offspring (juvenile), with emphasis on growth performance, biochemical composition and stress resistance to low oxygen, high ammonia and salinity. Aquac Nutr 20:557–565; doi:10.1111/anu.12109
Rohlf J, Sokal R (1995) *Statistical Tables*. 3rd ed. Macmillan: New York, EUA
Romagosa E, Bittencourt F, Boscolo WR (2012) Nutrição e alimentação de reprodutores. In: NUTRIAQUA: nutrição e alimentação de espécies de interesse para a aquicultura (Fracalossi DM, Cyrino JEP). Sociedade Brasileira de Aquicultura e Biologia Aquática:Florianópolis, SC. 167–184
Romano N, Zeng C (2017) Cannibalism of decapod crustaceans and implications for their aquaculture: A review of its prevalence, influencing factors and mitigating methods. Rev Fish Sci Aquac 25:42–69; doi:10.1080/23308249.2016.1221379
Rostagno HS, Albino LFT, Calderano AA, Hannas MI, Sakomura NK, Perazzo FG (2024) Tabelas brasileiras para aves e suínos - Composição de alimentos e exigências nutricionais. Editora Scienza
Roustaian P, Kamarudin MS, Omar H Bin, Saad CR, Ahmad MH (2001) Biochemical changes in freshwater prawn *Macrobrachium rosenbergii* during larval development. J World Aquac Soc 32:53–59; doi:10.1111/j.1749-7345.2001.tb00921.x
Roustaian P, Kamarudin MS, Omar H, Saad CR, Ahmad MH (2000) Amino acid composition of developing larval freshwater prawn *Macrobrachium rosenbergii*. J World Aquac Soc 31:130–136; doi:10.1111/j.1749-7345.2000.tb00708.x
Silvia CGP, Gaiotto JR, Ribeiro FAS, Nunes AJP (2012) Boas práticas de fabricação e formulação de rações para peixes. In: Nutriaqua: nutrição e alimentação de espécies de interesse para aquicultura brasileira. (Fracalossi DM, Cyrino JEP). Sociedade Brasileira de Aquicultura e Biologia Aquática:Florianópolis, SC. 295–346
Soares MP, Jesus F, Almeida AR, Domingues I, Hayd L, Soares AMVM (2020) Effects of pH and nitrites on the toxicity of a cypermetrin-based pesticide to shrimps. Chemosphere 241:125089; doi:10.1016/j.chemosphere.2019.125089
Soares MP, Jesus F, Almeida AR, Zlabek V, Grabic R, Domingues I (2017) Endemic shrimp *Macrobrachium pantanalense* as a test species to assess potential contamination by pesticides in Pantanal (Brazil). Chemosphere 168:1082–1092; doi:10.1016/J.CHEMOSPHERE.2016.10.100
Soares MP, Oliveira N, Rebelo D, Marcondes SF, Fernandes CE, Domingues I (2019) Cypermethrin-based formulation Barrage ® induces histological changes in gills of the Pantanal endemic shrimp *Macrobrachium pantanalense*. Environ Toxicol Pharmacol 67:66–72; doi:10.1016/j.etap.2019.01.014
Valenti WC (1993) Freshwater prawn culture in Brazil. WORLD Aquac ROUGE 24:30–30
Valenti WC, Hayd LDA, Vetorelli MP, Inez M, Geraldo E, Federal P (2011) Economic analysis of Amazon river prawn farming to the markets for live bait and juveniles in Pantanal, Brazil. Bol do Inst Pesca 37:165–176
Vercesi K, Hayd L (2015) Avaliação do número de ovos em diferentes estágios de desenvolvimento embrionário de *Macrobrachium pantanalense*. Bol do Inst Pesca 41:655–663
Weiss R, Klaus A, Hayd L, Schubart CD (2015) Interpreting genetic distances for species recognition: the case of *Macrobrachium amazonicum* and the recently described *M. pantanalense* (Decapoda, Palaemonidae) from Brazilian freshwaters. Crustaceana 88:1111–1126; doi:10.1163/15685403-00003471
Wickins JF, Lee DO (2002) Crustacean farming: ranching and culture. 2nd ed. (Lee WJD) Blackwell Science Ltd:Oxford

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