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Diets for *Macrobrachium acanthurus* breeders: Sperm evaluation†

Dietas para reprodutores de *Macrobrachium acanthurus*: avaliação espermática

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Abstract
The application of biotechnology in animal reproduction has enabled the production of young forms in both quantity and quality. Increasing the number of viable gametes produced by reproducers, among other factors, through an ideal diet, can ensure higher production. Therefore, the aim of this study was to determine the influence of three diets on the sperm survival of *Macrobrachium acanthurus*. To this end, 24 *M. acanthurus* males were used, distributed randomly and equally among treatments. Their diets were composed of 100% fresh food (fish and squid muscle - 14% protein), 100% dry feed (commercial feed - 50% protein) and a mixture of these diets containing 30% protein. Spermatophores were extracted through electrical stimulation every 15 days, and the controls consisted of spermatophores obtained directly from nature. No significant difference between diets was observed comparing shrimp and spermatophore weights. The 100% fresh diet provided the best sperm survival performance.
Keywords: Caridae; cinnamon shrimp; nutrition; sperm

Resumo
A aplicação de biotecnologias na reprodução animal tem possibilitado produzir formas jovens em quantidade e qualidade. O aumento da quantidade de gametas viáveis produzidos pelos reprodutores, mediante uma dieta ideal, entre outros fatores, pode garantir uma maior produção. Assim, o objetivo deste trabalho foi determinar o efeito de três dietas quanto à sobrevivência espermática de *Macrobrachium acanthurus*. Para tanto, 24 machos de *M. acanthurus* foram utilizados, sendo distribuídos ao acaso e de forma igual entre os

tratamentos. As dietas foram compostas por 100% de alimento fresco (músculo de peixe e lula - 14% de proteína), 100% de alimento seco (ração comercial - 50% de proteína) e uma mescla dessas dietas contendo 30% de proteína. Os espermatóforos foram extraídos por eletroestimulação a cada 15 dias, sendo o controle aqueles obtidos diretamente da natureza. Não houve diferença significativa entre as dietas quando comparado os pesos dos camarões e dos espermatóforos. A alimentação 100% fresca proporcionou o melhor desempenho para a sobrevivência espermática.
Palavras-chave: Caridae; camarão canela; nutrição; esperma

Introduction

Macrobrachium acanthurus is a freshwater shrimp native to Brazil and found throughout the American continent and the West Indies, exhibiting high cultivation potential and considered economically important, especially for the human populations that manually exploit this species[1-4].

Investments in shrimp production, management, nutrition, reproduction and, more recently, genetic improvements, have become necessary for shrimp cultivation development. Maintaining shrimp with low reproductive quality implies in high costs for aquaculture farmers. Some techniques to improve reproductive quality have been carried out for *Litopenaeus vannamei* and *M. rosenbergii*, such as gonode characteristic assessments or the evaluation of molecules functionally related to the reproductive process[5].

According to Browdy[6], Meumpol *et al.*[7] and Shailender *et al.*[8], shrimp breeder diet and quality directly interfere in gamete production, and are, thus, responsible for adequate larvae quality and paramount for culture success. In general, nutritional shrimp reproduction effects have been assessed mainly in females. However, males may also contribute to the limited success of certain native species in captive breeding activities[9-13].

In males, sperm quality can be used as a tool to evaluate dietary efficiency concerning reproduction and maturation, having been studied in *L. vannamei*[14], *L. setiferus*[15], *Penaeus monodon*[7,16,17], *Farfantepenaeus paulensis*[18] and *P. merguiensis*[19]. In freshwater shrimp, studies have been conducted by Samuel *et al.*[11] on *M. malcolmsonii,* and Pérez-Rodriguez *et al.*[20] on *M. americanum*.

Food management regarding shrimp maturation includes fresh food, such as squid, polychaetes, bivalves, crabs and fish, with or without the addition of commercial feed[6,21]. Due to the risk of disease transmission, crustacean tissues have been removed from captive shrimp diets, thus ensuring reproductive biosafety and not influencing reproductive performance[16].

According to Harrison[22] and Wouters *et al.*[23], the importance of fresh ingredients is due to their nutritional profiles, in particular the content and amount of certain

amino acids, lipid fractions and essential fatty acids, such as arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) acid, which crustaceans find easy to digest and are recognized as playing significant metabolic and physiological roles in peneid reproduction[24]. González-Baró and Pollero[25] reported the inability of *M. borelli* shrimp to synthesize ARA and EPA from other fatty acids, justifying the need for dietary supplementation.

In this context, the present study aimed to determine the effect of three diets on *M. acanthurus* sperm production.

Material and methods

A total of 24 adult *M. acanthurus* males were collected in January 2014 from the Sahy River (22° 56'S, 44° 01'W) and taken alive to the Marine Biology Station belonging to the Federal Rural University of Rio de Janeiro.

The shrimp were individualized in 42 L aquariums containing sandy substrates and shelters under constant aeration. The animals were then measured (cephalothorax length) with a digital caliper (0.01 mm) and body and spermatophore weights were obtained using an analytical balance (0.1 mg accuracy). Aquaria water was renewed every three days and temperature (23.1 ± 1.4 ° C), pH (7.0 ± 0.5) and dissolved oxygen (7.4 ± 0.5 mg/L) were monitored daily with a multiparametric equipment (Akso Produtos Eletrônicos, model AK 88). Ammonia (0.9 ± 1.0 ppm) and nitrite (0.2 ± 0.4 ppm) were measured every two days (Hanna Instruments Brazil, model HI 83203).

The animals were distributed into three treatments comprising eight repetitions, with each individual constituting an experimental unit, in order to obtain weight and length homogeneity. Each treatment consisted of a diet comprising commercial feed (dry food) for marine shrimp breeders and two fresh food items, squid *Loligo* sp. and croaker *Micropogonias furnieri* muscle. In treatment T1, only commercial feed was offered. In treatment T2, squid and fish muscle were mixed at a 1:1 ratio and processed in a meat grinder until obtaining a homogeneous mass. In treatment T3, one part of the crushed feed and two parts of the fresh food mass were used.

After preparing the food items, a 100 g sample of each diet was taken and sent to the UFRRJ Bromatology Laboratory for dry matter (% DM), crude protein (% CP), total lipid (% LT) and mineral content (% MM) determinations[26], displayed in Table 1. For crude energy determinations (Kcal/Kg) 0.5 g of the previously processed sample were analyzed using a isoperibolic calorimetric model 2000 IKA pump (0.001 ° C precision)[27].

Food was provided daily at 10 am and 5 pm, on a 10% live weight basis. The first food offer was only performed after extraction of the first spermatophores, via electrostimulation (6.0 volts), carried out 24 hours after animal conditioning. The first seminal materials comprised the controls, consisting of sperm produced in the natural environment. The remaining extractions occurred every 15 days, totaling four extractions carried out after the captive food supply began. The shrimp were also weighed during each of the four extractions but were measured again only at the end of the experimental period (60 days). Spermatophores were extracted from all animals, although only those from six

shrimps were used to count sperm survival, using semen smears stained with eosin-nigrosine, each constituting an experimental unit.

Table 1. Proximate composition on a dry matter basis of the experimental diets supplied to the evaluated shrimps and their crude energy levels.

	%				Kcal/Kg
	DM	CP	MM	TL	Energy
T1 Commercial feed	88.6	50.3	8.6	10.9	4,866
T2 Fish + Squid	16.7	14.5	1.1	1.2	5,281
T3 Commercial feed + Fish + Squid	47.8	30.3	4.3	5.5	5,140

Dry matter (DM), Crude protein (CP), Mineral Matter (MM) and Total lipids (TL).

Before each extraction procedure, the six animals that provided spermatophores for the spermatic survival analysis were randomly selected, with the rest extracted only to maintain the same experimental conditions. Only one spermatophore per shrimp was used and when two were extracted, one was chosen at random. The other two shrimp that were not extracted obligatorily provided seminal material for the subsequent extraction, so all animals contributed to sperm survival counts.

The data were submitted to the Shapiro-Wilk test to assess data normality. To verify significant differences, an analysis of variance (Anova On-Way) and Tukey's test were applied at a 5% significance level.

Results

Shrimp displayed a 3.9% cephalothorax length growth in the commercial feed treatment (24.0 ± 4.6 mm initial; 25.0 ± 3.8 mm final). Animals that consumed the fish and squid mixture exhibited a 0.9% growth rate (23.5 ± 2.9 mm initial; 23.7 ± 2.7 mm final), while shrimp from the treatment comprising a mixture of fresh food and commercial feed displayed an 8.5% growth rate (22.1 ± 2.2 mm initial; 24.0 ± 2.2 mm final). However, growth rates did not differ significantly between treatments.

Table 2 presents the effect of the food items on the assessed biometric parameters. Regarding shrimp weight and spermatophore weight, no significant difference between treatments during the different collections was noted, although shrimp weight fed with the commercial feed and fresh food mixture became higher with each extraction.

Regarding sperm survival, treatment T2 exhibited the best result, with an average of 80.1%, although no significant difference compared to T1 was noted (Table 2). However, when considering only the four captivity collections, survival increase was of 19.4% (T2) compared to the control, while the other treatments resulted in 6.1% (T1) and 5.7% (T3) decreases.

Concerning sperm production, 100% of the animals produced two spermatophores during collections. Mortality was only observed at the end of the experimental period, at 25% (two shrimp) for each treatment.

Table 2. Effect of feeding on shrimp and spermatophore weight and sperm survival in *Macrobrachium acanthurus*. (n=6)

Parameters	T	C0	C1	C2	C3	C4	Means
Shrimp weight (g)	T1	10.6±1.9	10.6±2.2	10.6±2.2	11.4±2.3	12.0±2.5	11.0±2.2 (p=0.99)
	T2	10.1±1.3	10.4±1.2	10.4±1.2	10.4±1.3	10.1±1.5	10.3±1.3 (p=0.99)
	T3	7.7±0.6	8.5±1.0	8.6±0.9	9.4±0.9	9.7±1.4	8.8±0.9 (p=0.60)
Spermatophore weight (mg)	T1	1.2±0.1	0.8±0.3	1.1±0.3	1.3±0.2	1.0±0.3	1.1±0.3 (p=0.81)
	T2	1.4±0.3	1.2±0.3	1.4±0.4	1.1±0.2	0.8±0.3	1.2±0.3 (p=0.55)
	T3	1.1±0.2	1.4±0.2	0.9±0.3	1.1±0.2	1.0±0.2	1.1±0.2 (p=0.62)
Sperm survival (%)	T1	80.4±5.8	67.5±6.8	68.5±8.1	85.1±3.0	81.0±4.7	$76.5±5.7^{ab}$ (p=0.16)
	T2	$69.4±3.9^{A}$	$76.4±4.9^{A}$	$81.0±5.0^{AB}$	$94.4±3.3^{B}$	$79.5±6.2^{AB}$	$80.1±4.7^{a}$ (p=0.01)
	T3	$76.7±3.9^{A}$	$65.4±6.8^{A}$	$75.7±5.3^{A}$	$86.2±4.5^{A}$	$62.1±5.5^{B}$	$73.2±5.2^{b}$ (p=0.02)

Means followed by equal letters in lines (uppercase) and columns (lowercase) do not differ significantly (p> 0.05) from each other. T: treatments; T1: commercial feed; T2: fish + squid and T3: commercial feed + fish + squid. C0 to C4: spermatophore extractions, with C0 being the control. Data are expressed as means ± standard error. The sample n for each treatment was of six spermatophores.

Discussion

Although *M. acanthurus* displays continuous reproduction according to Tamburus *et al.*[28], its reproductive peaks occur between December and January in southeastern Brazil. This is the ideal period for studies with breeders, since, according to Browdy[6] and Ogle[29], in natural conditions, environmental factors determine the existence of defined reproductive seasons, stimulating or inhibiting the reproductive process of a particular species, since gonadal maturation is under hormonal control, which is, in turn, controlled by environmental factors.

The water temperatures in farming environments are always higher than in the natural environment, affecting sperm survival, which, regardless of this factor, varies significantly among shrimp from the same population[30-32].

The average temperature in the present study is in accordance with the thermal amplitude determined by Díaz *et al.*[33], who mentioned a wide tolerance (15 to 38 °C) for *M. acanthurus*, indicating its ability to adapt to different regions[3], supporting conditions imposed by the environment without compromising sperm production, since the animals are exposed to a minimum of thermal stress in these temperature ranges. Therefore, the thermal variations imposed by the environment on the animals during the experimental period did not compromise the sperm production process.

Oxygen consumption in *M. acanthurus* is inversely proportional to weight, and larger animals require lower water oxygen concentrations[34,35]. According to Alves *et al.*[36],

oxygen concentrations should be higher than 5 mg/L, as in the present study. Ammonium and nitrite levels were also adequate (<1.5 ppm). Poor water quality, not observed herein, has been reported as an interfering factor in reproduction processes[32,37].

It is known that growth, even during the reproductive period, is continuous in crustaceans, although at a slower pace than in early life stages[38]. However, fatty acids are also responsible for freshwater shrimp growth[39]. Papa[40], when studying *M. amazonicum* males, reported that diets containing higher amounts of cholesterol inhibited body and testicular growth, as reported for other decapod species, which could justify the results reported for T2, in which the animals exhibited the lowest weight/size growth (0.9%), despite the highest sperm survival (19.4%). Ribeiro *et al.*[41] mentioned that dietary lipid levels can be low if they provide a sufficient amount of fatty acids, the requirements of which are distinct among crustacean species[25]. In this regard, Leelatanawit *et al.*[17], when feeding *P. monodon* with polychaetes, rich in fatty acids (ARA, EPA and DHA), reported increases in shrimp weight and size, as well as in spermatophore weight, from the third week.

Lipids are considered important sources of energy for crustaceans. However, diets containing high levels of these compounds may not contain adequate amounts of carbohydrates as a source of glucose, which is even more necessary in some cases, for example, for chitin synthesis, justifying a balanced diet[42]. Pérez-Rodríguez *et al.*[20] reported that carbohydrates present in dry food may have been essential for the recovery of sperm production in the assessed animals, which were maintained in captivity for a long period of time.

In addition to their importance for chitin production, carbohydrates, as well as glycoproteins, are present in seminal fluid and are essential for the sperm maturation process and maintenance of sperm viability[43-46]. Jeyalectumie and Subramonian[44] mentioned the reduction of chitin levels in *Scylla serrata* spermatozoa preserved at -4°C, indicating its consumption even at low temperatures. Thus, low concentrations of this fluid could compromise sperm survival during fertilization or cryopreservation processes, since this would not aid in injuries caused by the external environment, as well as in sperm energy supplies.

Previous studies with marine shrimp have demonstrated that spermatophore weights do not depend on the amount of contained sperm[12], but can be associated with shrimp age, body weight and structural components[10,20]. Thus, the lower weight of spermatophores found in T2 could be related to a lack of dietary carbohydrates in the fish and squid food items, although no significant differences were detected in relation to the other treatments. According to Sacristán *et al.*[47], decapod crustaceans present a great diversity of strategies for mobilizing their reserves, and the authors suggest that this is not related to type of food. Pérez-Rodríguez *et al.*[20] reported similar results to the present study for *M. americanum*, justifying that males require an insignificant amount of energy to produce sperm when compared to females and egg production.

Shailender *et al.*[8] and Memon *et al.*[19] mentioned that the inclusion of fresh food during the male maturation process is important to reduce the effects of natural spermatophore degeneration, as reported for other peneids. In addition to this factor,

according to Braga *et al.*[18], *F. paulensis* fed only commercial diets exhibited greater absence of seminal material and hyperglycemia in hemolymph. Thus, commercial feeds are not recommended for this species, and ideal diets should be composed of a mixture of fresh and dry food.

Due to the various studies carried out with shrimp belonging to the *Macrobrachium* genus, it is known that adult animals are preferentially carnivores and detritivores who accept all types of food with a certain animal protein content, thus able to be fed commercial feed in captivity[48,49]. However, when this diet alone is offered during the maturation period, production results are not satisfactory compared to fresh food. Therefore, commercial feed is indicated at a maximum composition of 50% for breeding peneids[7,14,50,51]. The same has been reported by Samuel *et al.*[11] for *M. malcolmsonii* males, who suggested the use of fresh food and commercial diets.

In the present study, the T3 diet containing commercial feed and fresh food (1:2) did not lead to satisfactory results regarding sperm survival, when compared to T2. However, Perez-Velazquez *et al.*[51] suggested that at least 25% of the maturation diet should be replaced with feed in order to supply vitamins and minerals[9], since these nutrients apparently prevent the ARA and EPA catabolism, as reported for *M. borelli*[25].

Weight loss and decreased sperm counts have been reported when *L. vannamei* were fed 100% fresh food[14,51], corroborating the results of the present study. However, further investigations are required to unravel the specific role of each nutrient in male reproduction processes.

Among organic food compounds, it has been suggested that proteins are more associated with spermatophore and sperm quality than lipids or carbohydrates, since proteins are related to the formation of the sperm spine, essential during the sperm fertilization process in Dendrobranchiata[15,52,53].

In their studies with male *L. setiferus* individuals, Goimier *et al.*[15] suggested that excess dietary protein can trigger stress, causing loss of sperm quality and decreased management in captivity[54]. High protein levels increase hemocyte concentrations in shrimp, preparing the animals for an immune defense. Thus, if the animals are stressed by internal ammonia, an immunological reaction can be activated as a response, affecting various physiological functions, including sperm production and quality, following the same mechanism as when shrimp are submitted to extreme temperatures[15,37]. However, these factors do not seem to interfere with *M. acanthurus*, as also reported by Memon *et al.*[19], since spermatophore production was maintained throughout the experimental period, without any significant difference in relation to the control for the diet containing the highest protein content.

Conclusions

The combination of an inert diet with a fresh diet resulted in greater relative shrimp growth and did not significantly alter spermatophore weight. Regarding sperm survival, the fresh diet led to significant results in relation to a natural environment diet. However,

the need for further studies regarding nutritional effects on freshwater shrimp sperm production and implications for reproductive processes is noted. The present study may also indicate the rusticity of this native species, of good sperm production, even without a specific diet.

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