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Reproductive performance and offspring quality in mud crab (*Scylla paramamosain*) broodstock fed different diets

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Abstract. A 2-month feeding trial was conducted to evaluate the reproductive performance and offspring quality of mud crab (*Scylla paramamosain*) females fed either a mixture of fresh food items (squid, shrimp, trash fish and *Artemia* biomass) or two experimental diets developed for penaeids. Before test initiation, mud crab females with an average individual wet weight of 200–300 g were acclimated for 2–3 days and reared together in one concrete tank of 2.0 × 0.5 × 8 m until spawning. After spawning, the spent spawners were unilaterally eyestalk ablated and randomly divided (20 animals/treatment) over three tanks of the same size and subjected to the dietary treatments. Spent spawners were used to eliminate the effect of feeding history.

There were only minor differences in reproductive performance between dietary treatments. No differences were observed in the duration of the latency period from eyestalk ablation to spawning. Fecundity was only marginally higher for the broodstock fed the control diet. Also egg quality seemed only slightly affected by the treatments. Egg hatching rates were slightly higher in crabs fed the formulated diets compared to those crabs fed the fresh diet. The only statistically significant difference ($p < 0.05$) observed however was in egg hatching rate between the control diet and diet A2. In contrast, the crabs fed the fresh diet produced stronger larvae as determined by a starvation test.

We therefore conclude that artificial diets resulted in reproduction success comparable to the use of fresh food. The nutritional composition of the artificial diets could however be improved in order to produce larvae of optimal quality. Based on our research findings, the protein level and n-3 HUFA level in the diet warrants further investigation in this respect.

Key words: Artificial diets, Broodstock feeding, Mud crab, *Scylla paramamosain*

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Introduction

Although mud crab farming in Indonesia has been practiced for several years and its production amounts to about 40% of total crab production (Directorate General of Fisheries 1999), all crab aquaculture production relies on wild caught seedstock as larval rearing has not yet reached a commercially viable level for stocking into farms. Development of a reliable seed production technique, including domestication of broodstock, is clearly critically important for sustainable growth of the industry.

Studies on crustacean broodstock nutrition began concertedly during the last decade with the growing demand for controlled reproduction in commercial facilities. However, reliable data on the nutrient requirements specific to maturation, reproduction and embryogenesis in crustaceans are scant and fragmentary (Harrison 1990). The importance of diet on gonadal maturation and spawning is well documented for certain crustacea especially for penaeid shrimp (Beard and Wickins 1980; Chamberlain and Lawrence 1981; Millamena et al. 1986; Sangpradub et al. 1994; Xu et al. 1994; Marsden et al. 1997) and fresh water prawn (De Caluwe et al. 1995; Cavalli et al. 1999). For mud crab the only available data on broodstock maturation diets at present is from research conducted by Millamena and Quinito (2000) and Millamena and Bangcaya (2001). Crustacean broodstock in captivity are generally fed with chopped fresh food, which has a high nutritional value and is generally regarded as superior to compound diets (*Penaeus indicus*, Cahu et al. 1995; *Litopenaeus vannamei*, Laufer et al. 1998). However, fresh food decays rapidly and easily deteriorates water quality (Sheen and Wu 1999). Moreover, problems with availability further restrict its usefulness as a regular diet. As a consequence, it is necessary to develop artificial feeds that fulfill the nutritional requirements of cultured species, minimize water fouling, are cost-efficient and produce prime quality seed. In addition, artificial broodstock diets form a necessary tool to study exact nutrient requirements. In this experiment, the nutritional value of a diet composed of a mixture of fresh food items was compared with two types of formulated diets.

Material and methods

Experimental conditions

The experiment was conducted at the crustacean hatchery of the Center for Brackishwater Aquaculture Development Center (CBAD), Jepara, Indonesia. Mud crab females with an average individual wet weight of 200–300 g were

purchased from local commercial collectors. Before test initiation, the crabs were acclimated for 2–3 days to the experimental rearing condition and reared together in one concrete tank of 2.0 × 5.0 × 0.8 m. The tank was provided with a 20 cm thick layer of mud as bottom substrate and several pieces of PVC tubes (3.0 inch diameter) of 20 cm long to serve as shelter in order to reduce cannibalism during moulting. The tanks were filled with filtered seawater to a depth of 60 cm and aerated. Seawater salinity was maintained at 30 ± 1 ppt and temperature at 28 ± 1 °C. The water in each tank was replaced 100% daily with fresh seawater. When renewing the water, uneaten food and dead animals were removed. Broodstock were fed a mixture of fresh food consisting of squid, shrimp, trash fish and *Artemia* biomass with the same ratio until they spawned. After spawning, the crabs were individually weighed, carapace width and length measured, tagged, and unilaterally eyestalk ablated. Tagging was done by engraving identification numbers on the carapace of the animals. The spent spawners were subsequently randomly divided over three concrete 8-m^3 tanks at a density of 20 females $tank^{-1}$ and subjected to the dietary treatments. Rearing conditions were the same as during acclimation.

Experimental diets

Dietary treatments consisted of a mixture of fresh food as control diet, and two types of formulated diets. The control diet was composed of an 80% mixture of shrimp, squid, trash fish with the same ratio and 20% *Artemia* biomass. Before feeding to the crab, *Artemia* biomass was prepared in the following manner. Agar was dissolved in boiling water and cooked gently for 2 min. *Artemia* were subsequently poured into it (28 g agar for 1 kg *Artemia*), and mixed thoroughly for 5 min, stuffed in Kurehalon plastic tubes, and again steamed for another 5 min. After cooling in air, these were directly used or stored in refrigerator until use.

The control diet was compared to two types of formulated diets; diet A1, based on Marsden et al. (1997) and diet A2, based on an experimental formulation for *Litopenaeus vannamei* broodstock (Wouters et al. 2002). Experimental diet compositions are presented in Table 1. The diets were prepared by weighing the dry ingredients and mixing thoroughly in a mixer (Hobart M D300T). The lipid sources were added drop by drop while the mixture was further blended to ensure homogeneity. Approximately 200 ml of distilled water was then added for each kg of this mixture. In order to form a firm dough the wet mixture was steamed without pressure for 15 minutes. After cooling, the dough was divided into small pieces and shaped manually into boluses with a diameter of approximately 2 cm. The soft wet pellets were

Table I. Ingredient composition of experimental diets (in g 100 g dry diet^{-1} basis)

Ingredients	Diet A1	Diet A2
Fish meal	–	8.15
Fish hydrolysate	–	3.72
Krill meal	–	3.72
Squid meal	43.7	28.95
Minced mussel	24.5	–
Calf liver	10.5	–
Artemia biomass	–	17.25
Wheat gluten	–	6
Soybean meal	–	5
Wheat flour	–	4
Dry selco[1]	4	–
Lecithin[2]	1	1
Cholesterol[3]	–	0.5
DHA oil[4]	–	1.06
Vitamin mix[5]	3	3
Vitamin C[6]	1	1
Vitamin E[7]	0.05	0.05
Choline chloride[8]	1	1
Mineral mix[9]	4	4
Binder[10]	6	6
Ethoxyquin[11]	–	0.02
Attractant[12]	–	1.5
Astaxanthin[13]	1.25	1.25
Corn starch	–	2.83
ß-carotene[14]	0.005	0.005

[1]INVE Aquaculture N.V., Belgium; [2]EMULPUR N, Lucas Meyer GmbH & Co, Germany; [3]Sigma C-8503; [4]INVE Aquaculture N.V., Belgium; [5]Kanazawa (1981); [6]Stay-C®, Roche, France; [7]dl-α-tocopherol-acetate, Federa N.V., Belgium; [8]Sigma C-1879; [9]Kanazawa (1981); [10]INVE Aquaculture N.V., Belgium; [11]Sigma E-8260; [12] INVE Aquaculture N.V., Belgium; [13]Carophyll® Pink, Roche, France; [14]Sigma C-9750

stored in a refrigerator until use. The crabs were fed the experimental diets twice a day (08.00 and 18.00 h) with the daily amount calculated as 15–20% and 3–5% of total crab biomass per tank for control and formulated diets, respectively.

Evaluation of broodstock performance, egg and larval quality

The reproductive performance of individual broodstock was followed over a 2-month period. Gonadal maturation was first checked one week after ablation and every three days thereafter. This was done through visual observation of gonads by pushing the first abdominal segment, which borders the carapace. Broodstock that presented yellow or orange gonads were considered mature. The number of females maturing and spawning and the period of time between eyestalk ablation and spawning were recorded.

Berried crabs were transferred to rectangular 2-m^3 tanks (one crab per tank) for egg incubation and hatching. Egg fertilization rate was estimated through microscopic examination on the fourth and sixth day after transfer to hatching tank by taking egg samples from different points of the egg mass. Fertilized eggs are pigmented and manifest eye formation, while unfertilized eggs are unpigmented and are uniformly dark or a black mass. Fertilization rate was estimated by comparing the number of fertilized eggs to the total fertilized and unfertilized eggs number in the sample (Millamena and Quinitio 2000). The number of crab delivering viable larvae was recorded for each treatment. The hatching rate of eggs and total number of zoea produced per female was calculated by counting triplicate 1-l samples from the hatching tank. Fecundity was expressed as the number of eggs (number of zoea and unfertilized eggs) per g body weight of female. All parameters were recorded for the first spawning only. Animals that spawned a second time during the experimental period were not considered.

The quality of the larvae was determined by observing their phototaxis response. After switching off aeration, weak or dead larvae that concentrated at the bottom of the tank were siphoned out and counted by taking triplicate samples. The number of larvae remaining in the hatching tank were also estimated from triplicate samples. The percentages of phototactic larvae was determined by comparing the number of remaining larvae in the hatching tank to the total number of larvae produced. Offspring quality was further evaluated by means of a starvation test. From each batch, 4 replicate groups of 100 mud crab larvae were starved in 1-l glass beakers under standardized conditions (31 ± 1 °C and 31 ± 1 ppt). Survival rate was monitored at 12 h intervals over a 120 h period.

Chemical analysis

Fresh food, formulated diets and larvae were analyzed for proximate composition according to standard methods (AOAC 1984). Total lipids of the diets and larvae were extracted according to Folch et al. (1957) modified by Ways and Hanahan (1964) using chloroform and methanol (2:1,v/v). Solvent

was evaporated under a stream of nitrogen. Prior to weighing, lipid extracts were dried overnight in a vacuum desiccator. Lipid extracts were redissolved in solvent mixture containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant at a concentration of 10 mg ml^{-1}, and used to determine the fatty acid profile of total lipid.

Fatty acid composition of total lipids was determined following the method of Christie (1989). Fatty acids were transesterified for 16 h at 50 °C using a mixture of sulfuric acid and methanol (1:100 by volume), and tricosanoic acid (23:0) as an internal standard. Fatty acid methyl ester (FAME) were extracted with hexane, dissolved in iso-octane and determined quantitatively with a Chrompack CP9001 gas chromatograph equipped with an autosampler. Injection was done on a very polar 50 m capillary column, BPX70, with a diameter of 0.32 mm and a layer thickness of 0.25 μm connected to a 2.5 m methyl deactivated pre-column. The carrier gas was H_2 and the detection mode was flame ionization detection (FID). The oven was programmed to rise the initial temperature from 8 5°C to 150 °C at a rate of 20 °C min^{-1}, from 152 °C to 174 °C at 0.7°C min^{-1}, from 174 °C to 180 °C at 10 °C min^{-1} and to stay at 180 °C for 2 min. Identification was based on a standard reference mixture (GLC 68B, NU-Chech Prep). Integration and calculation were done using the software program "Maestro" (Chrompack).

Statistical analysis

Data are presented as means ± standard deviation. Statistical significance of differences among treatments was determined using one-way analysis of variance. Tukey's multiple range test was applied to detect significant differences between means ($p < 0.05$). Percentage data were arc-sin transformed prior to analysis (Sokal and Rohlf 1995).

Results

Proximate and fatty acid composition of diets and mud crab larvae

The proximate analysis and fatty acid profile of the diets are presented in Tables 2 and 3. The control diet had a higher protein content (66.8%), but lower total lipid level (7.0%) compared to both formulated diets (40.4% and 17.9%; 43.3% and 15.6%, respectively). The ash content was also much lower in the control diet (11.4%) compared to the compounded diets (18.8–20.6%). Nitrogen-free extract content was similar among treatments (12.4–14.9%). The level of most fatty acids on a mg g DW^{-1} basis was substantially lower in the control diet compared to both formulated diets. The control diet was especially low in n-6 PUFA's (especially 18:2n-6), which resulted in an increased

Table 2. Proximate composition (%) of control and formulated diets for mud crab, *S. paramamosain* broodstock

Dietary treatment	Moisture%	% Dry matter basis				
		Crude protein	Crude fat	Crude fiber	Ash	N-free extract
Control	81.9(2.5)	66.8(2.4)	7.0(0.8)	nd	11.4(0.6)	14.9(0.9)
Diet A1	33.2(0.9)	40.4(0.6)	17.9(0.1)	8.7(0.2)	20.6(0.3)	12.4(0.2)
Diet A2	30.3(1.8)	43.3(2.2)	15.6(0.4)	8.9(0.3)	18.8(0.7)	13.3(0.5)

nd: not detected
Values in parentheses are standard deviation

n-3/n-6 ratio compared to the two formulated diets. In contrast, the absolute levels of the essential fatty acids EPA and DHA and the total n-3 HUFA level were much higher in both formulated diets than in the control diet.

Table 4 shows the biochemical composition of the mud crab larvae originating from the different dietary treatments. The differences in crude protein and essential fatty acid levels in the broodstock diets were reflected in the composition of the larvae produced. The higher protein level in the control diet resulted in a significantly higher level in the larvae produced from the broodstock fed this diet (38.12% versus 30.14–31.06%). Similarly, the EPA, DHA and total n-3 HUFA levels were lower in the larvae produced from the broodstock fed the control diet compared to the larvae produced from the broodstock fed both formulated diets.

Reproductive performance, egg and larval quality

Reproductive performance and the egg and larval quality parameters of the mud crab broodstock fed the different diets are presented in Table 5. In general, there was hardly any significant effect of dietary treatment on reproductive performance. All diets resulted in 100% maturation and spawning of the crab. The latency period from eyestalk ablation to spawning was very similar between treatments. Fecundity was highest for the control diet and lowest for diet A1, but no significant differences were observed. Average fecundity varied from 2.10 to 2.33 million eggs female^{-1}.

The fertilization rate was higher in the crab fed formulated diets (82.1–83.7%) than in the crab fed the control diet (71.1%), but no significant differences were observed. Egg hatching rate was significantly affected by dietary treatments. The crabs fed diet A2 produced eggs with a significantly better hatching rate (95.3%) compared to the control diet (89.2%). No significant difference of hatching rate was observed for the crab fed diet A1 (90.9%) compared to diet A2 (95.3%).

Table 3. Fatty acid profile of the experimental diets (in % of total fatty acids and mg g DW^{-1} of total lipid, average of duplicate analyses)

Fatty acids	Control[1]		Diet A1		Diet A2	
	%	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}
14:0	1.68(0.11)	0.47(0.03)	3.01(0.06)	2.41(0.05)	1.41(0.11)	0.82(0.06)
16:0	19.10(1.04)	5.12(0.28)	20.11(1.03)	16.87(0.87)	18.39(0.84)	11.24(0.51)
16:1n-7	2.63(0.15)	0.68(0.04)	3.24(0.11)	2.73(0.09)	1.49(0.05)	0.91(0.03)
18:0	9.13(0.27)	2.41(0.07)	6.42(0.25)	5.44(0.21)	6.32(0.52)	3.82(0.31)
18:1n-9	10.20(0.22)	2.80(0.06)	9.73(0.42)	7.48(0.32)	12.90(0.70)	7.74(0.42)
18:1n-7	2.98(0.12)	1.03(0.04)	2.21(0.11)	1.82(0.09)	2.03(0.15)	1.22(0.09)
18:2n-6	6.23(0.07)	1.70(0.02)	15.44(0.40)	12.49(0.32)	22.30(0.45)	13.41(0.27)
18:3n-3	1.58(0.12)	0.42(0.03)	2.39(0.04)	2.01(0.03)	2.88(0.14)	1.80(0.09)
18:4n-3	1.13(0.09)	0.33(0.03)	1.02(0.04)	0.79(0.03)	0.30(0.04)	0.19(0.03)
20:1n-9	0.55(0.03)	0.24(0.01)	3.28(0.07)	2.72(0.06)	1.31(0.05)	0.82(0.03)
20:4n-6	5.08(0.16)	1.31(0.04)	2.09(0.05)	1.69(0.04)	1.89(0.09)	1.22(0.06)
20:5n-3	7.13(0.26)	1.87(0.07)	6.61(0.22)	5.48(0.18)	8.14(0.35)	4.91(0.21)
22:1n-9			3.03(0.09)	2.43(0.07)	0.32(0.03)	0.49(0.04)
22:5n-6	1.88(0.15)	0.49(0.04)	0.64(0.05)	0.52(0.04)	0.70(0.07)	0.52(0.05)
22:5n-3	0.68(0.03)	0.22(0.01)	1.43(0.02)	1.19(0.02)	1.43(0.05)	0.82(0.03)
22:6n-3	20.30(0.41)	5.40(0.11)	14.88(0.48)	12.42(0.41)	13.54(0.25)	8.21(0.15)
Σn-6 PUFA	15.15(0.53)	4.01(0.14)	18.47(0.71)	14.80(0.57)	24.79(0.88)	15.22(0.57)
Σn-3 PUFA	30.98(1.06)	8.20(0.28)	28.37(1.49)	22.79(1.49)	27.11(1.07)	16.53(0.65)
Σn-3 HUFA[2]	27.86(0.74)	7.49(0.20)	23.89(1.01)	19.81(0.84)	23.48(0.75)	14.32(0.48)
Σn-3/Ón-6	2.05(0.07)	2.05(0.11)	1.54(0.04)	1.54(0.05)	1.09(0.08)	1.09(0.01)
Total lipid	6.49(0.28)	64.89(2.75)	16.54(0.11)	165.42(1.07)	14.71(0.28)	147.07(2.79)

[1]Control diet is composed of 80% mixture of shrimp, squid, trash fish and 20% *Artemia* biomass; [2]Sum of n-3 ≥ 20:3n-3. Values in parentheses are standard deviation

Table 4. Biochemical composition of mud crab (*S. paramamosain*) larvae produced from broodstock fed different diets

Nutrient	Broodstock diet		
	Control	Diet A1	Diet A2
Crude protein (%)	38.12(1.93)^{a}	30.14(1.42)^{b}	31.06(2.74)^{b}
Total lipid (%)	6.29(0.13)^{b}	9.71(0.37)^{a}	9.47(0.26)^{a}
20:5n-3 (EPA, mg g DW^{-1})	3.97(0.17)^{b}	4.58(0.10)^{a}	4.11(0.14)^{ab}
22:6n-3 (DHA, mg g DW^{-1})	4.08(0.23)^{b}	4.98(0.03)^{a}	4.32(0.07)^{b}
Σn-3 HUFA (mg g DW^{-1})	8.85(0.08)^{c}	11.46(0.11)^{a}	9.72(0.03)^{b}

Values in the same row with different superscript are significantly different ($p < 0.05$)
Values in parentheses are standard deviation

Table 5. Reproductive performance, egg and larval quality characteristics of mud crab (*S. paramamosain*) broodstock fed different diets

Parameters observed	Dietary treatments		
	Control	Diet A1	Diet A2
No. of females	20	20	20
Maturation (% of total females)	100	100	100
Spawning (% of total females)	100	100	100
Hatching (% of total females)	100	100	100
Latency period (days)	21.8(1.0)	22.8(4.0)	21.3(2.8)
Incubation time (days)	9.0(0.8)	8.6(0.9)	8.8(1.0)
Fecundity (No. of eggs g BW^{-1})	7687(1812)	7321(1553)	7410(1608)
Egg fertilization rate (%)	71.1(12.1)	82.1(11.4)	83.7(13.3)
Egg hatching rate (%)	$89.2(2.2)^{b}$	$90.9(3.8)^{ab}$	$95.3(1.7)^{a}$
Zoea production (larvae $female^{-1} \times 10^{6}$)	2.07(0.55)	1.91(0.31)	2.18(0.38)
Phototaxis larvae (% of total larvae)	99.5(0.3)	96.8(3.0)	97.5(1.5)

Values in the same row with different superscript are significantly different ($p < 0.05$)
Values in parenthesis are standard deviation

Likewise, larval quality also seemed affected by the dietary treatments. Figure 1 shows the average survival in time during starvation. Over 90% of the larvae from all treatments could survive starvation for 24 hours. No significant difference was observed among the treatments during this period. From 36 hours onwards a significantly higher survival was however observed for larvae originating from broodstock fed the control diet (66.0% at 60 h, 52.5% at 72 h, and 45.0% at 84 h) compared to those fed diet A1 (49.0% at 60 h, 20.3% at 72 h, and 0% at 82 h) and diet A2 (45.5% at 60 h, 18.0% at 72 h, and 0% at 82 h). All larvae produced from the broodstock fed formulated diets died within 84 hours of starvation, whereas around 18.5% of the larvae produced from the broodstock fed the control diet could survive up to 120 hours of starvation.

Discussion

Research on nutrient requirements for broodstock maturation relies greatly on formulated diets. Also for commercial applications, artificial diets are preferred. These artificial diets offer many advantages compared to fresh feed, including a reliable supply, minimal preparation time and known nutrient content. Moreover, they offer the opportunity to orally administer drugs such as hormones or supplementary vitamins (Marsden et al. 1997). Various

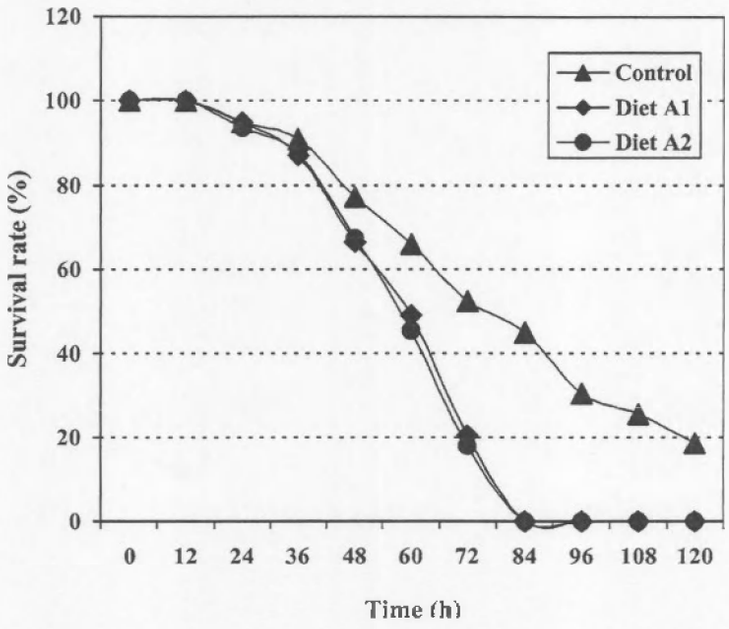

Figure 1. Survival of mud crab larvae under starvation conditions.

authors have successfully used dry artificial broodstock diets at a 50% substitution level of the total feeding regime (Primavera et al. 1979 and Millamena et al. 1986 for *Penaeus monodon*; Nascimento et al. 1991 and Wouters et al. 2002 for *Litopenaeus vannamei*). Similarly, for mud crab, Millamena and Quinito (2000) reported that provision of a formulated diet in combination with natural food results in improved consistency in reproductive performance of mud crab broodstock. The sole use of artificial diets on the other hand has only met variable success. Galgani et al. (1989a, b) and Marsden et al. (1997) reported however that better results on reproductive performance were obtained when shrimp broodstock were fed solely (100%) artificial diets containing minced fresh food. The results of our study suggest that although it was possible to successfully induce mud crab females to mature and spawn with the sole use of formulated diets, the artificial feeds tested here did not improve reproductive performance and even lead to a decline in the quality of the larvae produced.

In our study the formulated diets resulted in a higher hatching rate compared to the control diet. It might be that the relatively low lipid and n-3 HUFA levels in the control diet are responsible for this. Published work on dietary lipid and essential fatty acids requirements of crustacean broodstock

are very scant. Bray et al. (1990) found that for *Penaeus stylirostris* broodstock a dietary lipid level of 11.1% as opposed to 7.8% and 13.9% resulted in the highest number of nauplii per spawn and the highest protozoea length. However, Wouters et al. (2001) reported that dietary lipid levels above 9% retarded ovarian maturation of *Litopenaeus vannamei*. These authors state that broodstock diets containing very high dietary lipid levels may negatively affect ingestion rate and hence reproduction success. In our study however, the high total lipid levels in the artificial diets (15–17%) did not lead to a decrease in broodstock performance. The differences in hatching rates between treatments could also be explained by the differences in n-3 HUFA levels between diets. The lower total lipid level in the control diet resulted in relatively lower n-3 HUFA levels in this diet compared to both artificial diets. This is in line with the results of Millamena (1989) who reported that dietary n-3 HUFA could improve the hatchability of eggs in *Penaeus monodon*. Alava et al. (1993) obtained retarded ovarian development in *Marsupenaeus japonicus* fed a HUFA free-diet. Cahu et al. (1994) observed a decline in the spawning rate of *L. vannamei* when the broodstock diet was deprived of HUFA and phospholipids. Wouters et al. (1999) reported for *L. vannamei* fed with enriched *Artemia* biomass, that after replacing the *Artemia* enrichment product by coconut oil (free of HUFA and cholesterol), a decrease in egg fertilization, repeated spawns and egg production per female was observed, yet the maturation frequency was not affected.

Despite the lower hatching rate, broodstock fed fresh food produced stronger larvae as determined by the starvation test. This might be due to the fact that the fresh food contained higher levels of other essential nutrients, such as protein which apparently played a more crucial role during starvation. In this respect, the higher protein level in the control diet, which is reflected in the larval composition, might be important.

Conclusions

The study confirms the importance of the nutritional quality of the broodstock diet for reproduction and larval quality of mud crab, *S. paramamosain*. In that respect, more extensive research has to be done to determine the effects of other essential nutrients such as dietary protein level and quality, HUFA levels, and micro nutrients on reproductive performance.

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