Aquaculture Nutrition **2002 8;** 249–256

Experimental broodstock diets as partial fresh food substitutes in white shrimp *Litopenaeus vannamei* B.

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Abstract

In the first experiment, conducted in a research facility, *Litopenaeus vannamei* broodstock were fed either a 100% fresh food control treatment (FRE, consisting of frozen squid, oyster, mussel and enriched *Artemia* biomass in a 2.3:1.4:1.3:1 dry matter ratio) or one of the two treatments in which 50% [dry matter (DM)] of the fresh food was substituted with experimental artificial diets: a dry diet based on freeze-dried *Artemia* biomass (ART) and a control dry diet (CON). In the second experiment, conducted in a commercial hatchery, shrimp broodstock were fed either a fresh ration (FRE, consisting of frozen squid, polychaetes and enriched *Artemia* biomass in a 2.5:1.5:1 DM ratio) or the same experimental artificial diets (ART and CON) replacing 50% of the DM by elimination of polychaetes and *Artemia* biomass. In experiment 1 treatments CON and ART produced better results ($P = 0.05$) than treatment FRE in terms of spawn performance and egg production per female. In experiment 2 no differences were detected among treatments FRE and CON whereas treatment ART performed better ($P = 0.05$) in terms of spawning, egg production per female and spermatophore quality. Broodstock survival and offspring quality did not differ between treatments in either experiment.

KEY WORDS: diets, maturation, nutrition, reproduction, shrimp

Received 23 February 2001, accepted 8 November 2001

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Introduction

Diet is a factor that influences the reproductive performance and offspring quality of penaeid shrimp broodstock (Harrison 1990). In captive conditions, hatchery managers rely on fresh or fresh-frozen food to ensure optimal reproductive output. Fresh food items are generally wild-caught marine organisms selected according to their availability and fed in mixed ratios to increase the chances of meeting the nutritional requirements of the broodstock shrimp (Bray & Lawrence 1992; Pinon 2000). The most commonly used fresh food organisms are molluscs (squid, clam, mussel), crustaceans (shrimp, *Artemia* biomass) and marine polychaete worms (bloodworms) (Harrison 1997). The disadvantages of such fresh food organisms include high costs, fluctuation in availability, inconsistent nutritional value, need for frozen storage, water fouling, lack of potential for improvement, and increased risk of transmission of pathogenic bacteria and viruses (Harrison 1990, 1997).

Artificial dry diets are the most logical choice to solve the problems associated with fresh food. They have a generally stable cost, constant availability, constant and controlled nutritional value, long shelf-life, easy use and low risk of contamination. In addition, essential nutrients, hormones and therapeutics can easily be added. Unfortunately, artificial dry diets have not performed as well as fresh diets. Consequently, in commercial hatcheries in the western hemisphere only around 16% of the total feeding regime is constituted by an artificial broodstock diet (Wouters *et al.* 2000). The development of suitably performing artificial dry diets for shrimp broodstock is thus a research priority.

The present study evaluates the effect on wild white shrimp *Litopenaeus vannamei* B. of 50% fresh food substitution with two experimental artificial diets in terms of reproductive performance, spermatophore quality and offspring quality.

A previous study (Wouters *et al.* 1999) demonstrated that supplementation of a fresh food broodstock diet with fresh-frozen *Artemia* biomass improved *L. vannamei* reproductive output and larval quality. Spawners that received *Artemia* biomass showed higher survival percentages and better ovarian maturation, spawning and repeated spawning performance. Moreover, the spawners receiving *Artemia* biomass produced better offspring in terms of egg hatch, larval survival and larval length. *Artemia* biomass is collected from salt lakes, salinas, man-managed pond productions and intensive culture systems for use in shrimp and fish hatcheries (Lavens & Sorgeloos 1996). With the increasing threat of viral pathogens worldwide, seriously affecting hatchery and grow-out production in the shrimp industry, the need emerges for improved hygiene and strict biosecurity (Browdy 1998). This includes the use of dry feeds that are free of pathogens. The process of making a dry extruded broodstock diet – preconditioning, extrusion and drying (Harper 1988) – will indeed eliminate viable bacteria and viruses (Chris Dinneweth, INVE TECHNOLOGIES N.V., Baasrode, Belgium) because of the heat treatment (Chang *et al.* 1998) and the extraction of water. Several studies in Thailand showed that the processing temperatures and drying used for preparation of shrimp head meal and the temperatures for pellet production are sufficient to inactivate any white spot virus or yellow head virus that might be present (Flegel 2001). Although the use of fresh-frozen *Artemia* biomass has not been associated with an increased risk of infections, hatchery managers prefer dry diets to natural ingredients. Processing this *Artemia* biomass into a meal and incorporating it into an extruded broodstock diet could help hatchery managers to reduce the risk of transmitting pathogens to the shrimp broodstock (Harrison 1990, 1997). The question remains if the beneficial effects of frozen *Artemia* biomass on shrimp reproductive performance are maintained after these processing steps. It was as such an additional aim of this study to verify this.

Materials and methods

Experiment 1: Research facility

Animals Wild *L. vannamei* were caught by artisanal fishermen on the Ecuadorian coast between Ayangue and Olón (Guayas Province) and brought to the CENAIM research facilities in San Pedro. Upon arrival animals were treated against filamentous bacteria, fungal and protozoan infections with formalin according to the method described by Simon (1982) and kept in maturation tanks. Once enough animals were available for the experimental work, the animals were acclimated to the experimental conditions for a period of 2 weeks during which they were fed on the different experimental diets described below.

Experimental conditions Unilateral eye-stalk ablation was applied to the females by cutting and pinching, and the intact eye-stalk was marked with numbered rings to allow individual monitoring. Each tank was stocked with 40 females and 45 males. Culture conditions, daily routine and monitoring of evaluation parameters related to broodstock performance and offspring quality were as described by Wouters *et al.* (1999). Oval-shaped (5×3 m; 19.6 m^2) black fibreglass maturation tanks were used, supplied with sand-filtered and UV-treated sea water from a reservoir tank. Water was exchanged at a rate of 250% daily to keep water quality optimal and similar in all tanks. Water temperature in the maturation tanks was 29.4 ± 0.3 °C, salinity 32.7 ± 0.8 g L^{-1}, and pH 8.4 ± 0.1. A timer-controlled, inverted photoperiod of 14 h light:10 h dark was adopted, with gradual transition between light and dark hours. Mated females were transferred to individual 300-L black spawning tanks, and from each spawn the eggs were hatched out in 20-L buckets. Nauplii were collected after phototactic selection and stocked in 1-L bottles at a density of 100 L^{-1} and a temperature of 29 °C until metamorphosis to zoea 1 (Z1). The hatching percentage was estimated by concentrating the viable nauplii in a 10 L bucket and counting five subsamples. The percentage of egg fertilization was determined by the presence of a double membrane and/or embryonic development. Zoea 1 length was measured with a profile projector on samples of 30 zoea each. Spermatophore quality was based on sperm count and spermatophore weight (according Alfaro & Lozano 1993).

Dietary treatments During the acclimation period a feeding rate of 2.1% day^{-1} dry matter (DM) of the total shrimp biomass was applied. After ablation the feeding rate was increased to 3.15% day^{-1} DM. One dietary treatment consisted of a feeding regime of frozen fresh food containing squid, oyster, mussel and enriched *Artemia* biomass in a 2.3:1.4:1.3:1 ratio (DM) and is denominated 'FRE'. The enriched *Artemia* biomass was ongrown *Artemia* harvested from salt evaporation ponds at San Francisco Bay (USA) and enriched with menhaden fish oil and astaxanthin according to the standard bio-encapsulation method used by San Francisco Bay Brand Inc (CA, USA). In two additional dietary treatments, 50% DM of the fresh food was substituted with experimental dry diets. In these latter treatments, the enriched *Artemia* was completely substituted, while squid, oyster and mussel were fed in reduced quantities. The two experimental dry diets were formulated to be isolipidic and iso-nitrogenous (Table 1): diet CON (control diet), and diet ART (diet containing 33% *Artemia* meal).

The *Artemia* meal was also a SFBB product provided to us by INVE Americas Inc (Salt Lake City, UT, USA). This product was freeze-dried and ground through a 500-µm screen. All the ingredients (Table 1) were mixed with 45% (w/w) warm water (100 °C) and the resulting dough was heated and pressed through the 3-mm orifice die of a semi-industrial meat grinder. All holes but one of the die were blocked to increase pressure. The strands were dried in a ventilated oven at 60 °C for 2 h and kept in sealed nitrogen-flushed plastic bags at –20 °C until use. Each treatment was fed to a single broodstock tank for 70 days.

Table 1 Composition of the artificial broodstock diets fed to *Litopenaeus vannamei* (% dry matter) in experiments 1 and 2

	CON	ART	
	Experiment 1 & 2	Experiment 1	Experiment 2
Ingredients			
Squid meal[1]	35	19	28
Artemia meal[2]	0	33	20
Fish meal[3]	15.91	7.98	9.79
Krill meal[4]	5	3	3
Fish hydrolisates[4]	5	3	3
Wheat gluten[5]	6	6	6
Soybean meal[5]	5	5	5
Wheat meal[5]	4	4	4
Corn starch[6]	14.535	9.565	11.755
Fish oil[7]	1.16	1.06	1.06
Egg lecithin[8]	1	1	1
Cholesterol[9]	0.5	0.5	0.5
Vitamin mix[10]	2.28	2.28	2.28
Mineral mix[11]	2.1	2.1	2.1
Atractant[1]	1.5	1.5	1.5
Binder[12]	1	1	1
Etoxiquin[12]	0.015	0.015	0.015
Formulated composition			
Moisture	6	6	6
Protein	52	52	52
Lipid	10.5	10.5	10.5

[1] Rieber & Son, Bergen, Norway.
[2] Inve Americas Inc., Salt Lake City, UT, USA.
[3] CIPSSA, Puerto Montt, Peru.
[4] Profish SA, Santiago, Chile.
[5] Alimentsa, Guayaquil, Ecuador.
[6] Sumesa, Guayaquil, Ecuador.
[7] Pronova Biocare, Oslo, Norway.
[8] Lucas Meyer, Hamburg, Germany
[9] Sigma, St. Louis, MO, USA.
[10] Vitamin mix (mg kg^{-1} diet): ascorbic acid, 1000; biotin, 5; Ca pantothenate, 500; calciferol (D3), 12.7; choline, 3500; cyanocobalamine (B_{12}), 0.3; folic acid, 15; inositol, 4000; menadione (K3), 40; niacine, 750; p-amino benzoic acid, 100; pyridoxine HCl, 120; riboflavin (B_{12}), 200; thiamine, 120; vitamin A palmitate, 67; α-tocopherol, 400.
[11] Mineral mix (mg kg^{-1} diet): cobalt chloride, 0.249; copper sulphate, 7.7; Fe citrate, 624; KH_2PO_4, 7998; KIO_3, 0.747; manganese sulphate, 40; sodium phosphate, 6258; sodium selenate, 0.249, zinc sulphate, 324.
[12] Nutri-Ad International, Kasterlee, Belgium.

Experiment 2: Commercial facility

Animals Wild male *L. vannamei* were caught near San Pablo (Guayas Province, Ecuador) and brought to the commercial hatchery facilities of Granjas Marinas (El Rosario S.A) in Barandúa. Wild female *L. vannamei* were caught in the Esmeraldas Province and transported to Barandúa by truck, with animals placed in individual nets in insulated tanks with oxygenated and cooled sea water. These animals were kept in rectangular cement tanks for approximately 1 week before eyestalk-ablation and transfer to the maturation tanks.

Culture conditions Female spawners were ablated and ringed as described in experiment 1. The culture conditions in this test were those routinely used by Granjas Marinas: round 7-ton tanks made of fibreglass, 50 females and 50 males per tank, 300% daily water exchange with sea water of approximately 29 °C, two air stone diffusers per tank, natural photoperiod. Tank bottoms were siphoned once daily. Gravid females were sourced in the evening and transferred to individual spawning tanks. Upon spawning, eggs were concentrated in harvesting buckets and subsamples were taken for egg counts and further estimation of offspring quality according to the method described in experiment 1. As broodstock replacement is a continuous process in commercial hatchery applications, every newly introduced female was marked and the monitoring of its reproductive performance started after 2 weeks, over a period of 50 days. In total, 107 females were monitored in each treatment. Each treatment was fed to a broodstock tank for 80 days.

Dietary treatments A feeding rate of approximately 4% day^{-1} DM was applied in all treatments. One dietary treatment was the normal feeding regime of this hatchery (FRE) and consisted of frozen fresh squid, polychaetes from Maine, USA (*Glycera dibranchiata*) and enriched *Artemia* biomass in a 2.5:1.5:1 ratio (DM).

As in experiment 1, 50% DM of the fresh food was replaced with experimental artificial diets CON and ART. In these treatments, the polychaetes and the enriched *Artemia* were substituted completely, while squid was fed in the same quantity as in the FRE treatment. Formulation and preparation of the artificial diets were the same as in experiment 1, except that diet ART contained 20% DM *Artemia* meal instead of 33% (Table 1).

Statistical analyses

Because of system limitations in the experimental set-up, each dietary treatment was tested in one tank without replication. Individually tagged animals or spawns were taken as treatment replicates for statistical analyses. This is the most commonly used statistical procedure applied in shrimp reproduction trials (e.g. Browdy *et al.* 1989; Bray *et al.* 1990; Robertson *et al.* 1991; Menasveta *et al.* 1994; Xu *et al.* 1994; Cahu *et al.* 1995; Cavalli *et al.* 1997; Naessens *et al.* 1997; Wyban *et al.* 1997; Wouters *et al.* 1999). Possible effects caused by individual variation among experimental animals were reduced by randomly stocking the tanks and previous research has demonstrated that between-tank variability is minor (Luis Gómez, CENAIM-ESPOL Foundation, unpublished data). Infertile spawns were not considered. When necessary, data expressed in percentage or fractions were asin√ transformed to obtain normal distributions, although unadjusted means are presented. One-way ANOVA (Mead *et al.* 1993) was applied for statistical analysis (STATISTICA, Statsoft). An ANCOVA was applied in experiment 2 for the number of eggs produced per female, with number of spawns as covariate. Chi-square (Mead *et al.* 1993) was used for evaluating the number of spawns, the number of females with at least one spawning event, and the number of females with repeat spawn performance. Distribution-free parameters were not analysed statistically. To allow statistical processing of survival data, each female was assigned a calculated percentage value, termed 'useful lifetime', which corresponds to the number of days survived relative to the total number of days of the experiment (postablation). Reference to significant differences are at the 5% level.

Results

Female performance

The results of experiments 1 and 2 are summarized in Tables 2 and 3, respectively. Useful lifetime percentages were low in both experiments and did not differ significantly among treatments. The daily maturation frequency was significantly higher in treatment CON than in the remaining treatments (only evaluated in experiment 1). In experiment 1, the spawn frequency and the total number of spawns were higher in the treatments that received artificial diets (CON and ART) as compared with treatment FRE. This relation was also held true with significant differences for repeat spawn performance. The number of eggs produced per

Table 2 Reproductive performance of females, spermatophore quality and offspring quality of *Litopenaeus vannamei* shrimp in experiment 1 (research facility) as a function of dietary treatment. Mean ± SD are presented

	Dietary treatments					
	FRE	*n*	CON	*n*	ART	*n*
Female performance						
Useful lifetime (%)	49.0 ± 16.6^a	40	46.0 ± 16.9^a	40	42.1 ± 20.7^a	40
Maturation per female per day	0.114 ± 0.051^a	57	0.142 ± 0.064^b	57	0.121 ± 0.059^a	57
Spawns per day per female[1]	0.021 ± 0.012	40	0.043 ± 0.023	40	0.040 ± 0.025	40
Total spawns	22		60		50	
No. of females with ≥ 1 spawn	18^a		26^a		23^a	
No. of females with ≥ 2 spawns	3^a		17^b		14^b	
Fecundity (eggs per spawn × 10^3)	181.6 ± 76.0^a	22	213.5 ± 67.8^a	60	230.8 ± 80.1^a	50
Eggs per female	226.8 ± 132.8^a	40	480.5 ± 296.7^b	40	501.7 ± 449.2^b	40
Spermatophore quality						
Sperm count (10^6)	17.38 ± 12.89^a	37	15.57 ± 8.84^a	28	21.29 ± 14.82^a	36
Spermatophore weight (g)	0.068 ± 0.022^a	37	0.074 ± 0.023^a	28	0.076 ± 0.026^a	36
Offspring quality						
Egg diameter (mm)	0.270 ± 0.003^a	22	0.271 ± 0.006^a	60	0.271 ± 0.005^a	50
Egg fertilization (%)	51.6 ± 48.3^a	22	52.6 ± 47.7^a	60	69.2 ± 43.6^a	50
Hatch (%)	39.5 ± 29.9^a	22	38.4 ± 32.6^a	60	52.9 ± 29.3^a	50
Deformed nauplii (%)[1]	3.8 ± 8.0	18	3.3 ± 5.1	54	5.1 ± 6.6	45
Larval survival (%)[1]	56.4 ± 35.3	18	66.8 ± 28.4	54	67.1 ± 24.0	45
Zoea length (μm)	908.6 ± 84.6^a	18	901.9 ± 54.4^a	54	903.6 ± 43.9^a	45

[1] Distribution-free data were obtained, for which no statistical analysis was applied.
Means with different superscripts in the same row are significantly different ($P \le 0.05$).

Table 3 Reproductive performance of female, spermatophore quality and offspring quality of *Litopenaeus vannamei* shrimp in experiment 2 (commercial facility) as a function of dietary treatment. Means ± stdev are presented

	Dietary treatments					
	FRE	*n*	CON	*n*	ART	*n*
Female performance						
Useful lifetime (%)[1]	40.5 ± 42.4	107	44.2 ± 43.4	107	46.7 ± 41.2	107
Spawns per day per female[1]	0.057 ± 0.437	107	0.040 ± 0.189	107	0.051 ± 0.227	107
Total spawns	36		39		53	
No. of females with ≥ 1 spawn	25[a]		25[a]		28[a]	
No. of females with ≥ 2 spawns	8[a]		7[a]		17[b]	
Fecundity (Eggs per spawn × 10^3)	195.9 ± 89.4[a]	36	192.2 ± 88.3[a]	39	231.2 ± 109.6[a]	53
Eggs per female	282.1 ± 187.5[a]	107	293.2 ± 211.8[ab]	107	452.2 ± 200.4[b]	107
Spermatophore quality						
Sperm count (10^6)	6.23 ± 5.69[a]	20	7.72 ± 4.16[a]	20	12.02 ± 6.24[b]	20
Spermatophore weight (g)	0.0490 ± 0.148[a]	20	0.0461 ± 0.206[a]	20	0.0768 ± 0.226[b]	20
Offspring quality						
Egg fertilization (%)	75.2 ± 30.6[a]	36	75.9 ± 27.0[a]	39	83.2 ± 24.1[a]	53
Hatch (%)	72.5 ± 33.1[a]	36	74.7 ± 27.5[a]	39	81.1 ± 25.7[a]	53
Deformed nauplii (%)[1]	0.9 ± 0.3	36	1.0 ± 0.1	39	0.9 ± 0.2	53
Larval survival (%)	77.1 ± 20.8[a]	36	77.8 ± 19.4[a]	39	69.2 ± 20.2[a]	53

[1] Distribution-free data were obtained, for which no statistical analysis was applied.
Means with different superscripts in the same row are significantly different ($P \leq 0.05$).

female was also significantly higher in treatments CON and ART as compared with treatment FRE. In experiment 2, the spawn frequency was equal in treatments FRE and ART, but lower in treatment CON, while the total number of spawns and the number of females with repeat spawn performance were equal among treatments FRE and CON, but higher in treatment ART. This difference was statistically significant for the number of females with two or more spawning events. Furthermore, in experiment 2 the number of eggs produced per female in treatment ART was significantly higher than in treatment FRE, but not significantly different from treatment CON. Fecundity, or the number of eggs released per spawning event, was not affected by dietary treatment in either experiment.

Spermatophore quality

In experiment 1 sperm count and spermatophore weight did not significantly differ among treatments. In experiment 2 these parameters were significantly higher in treatment ART than in the remaining treatments. Sperm counts were generally higher in experiment 1 than in experiment 2.

Offspring quality

Offspring quality parameters were not affected by dietary treatment in any of the experiments. In general, the quality of the offspring produced in experiment 2 was higher than in experiment 1.

Discussion

Mortality rates were high throughout both experiments, resulting in useful lifetime percentages below 50%. It is known that adult shrimp are very sensitive to stress (Bray & Lawrence 1992; Browdy 1992; Mosquera 1999). For example, Naessens *et al*. (1997) report 50% mortality of wild *L. vannamei* during acclimation to culture conditions. However, in the present study, stress conditions were reduced to a minimum by improving transport from the beach to the hatchery facilities (shorter transport time, cooling of the transport tanks, placing the reproducers in small individual nets), careful handling and avoiding noise. From personal experience it seems that high mortality rates are almost inevitable with wild reproducers of the species *L. vannamei*. In agreement, high female mortalities are reported as a result of tank transfers and sourcing of *L. vannamei* (Galgani & AQUACOP 1989; Naessens *et al*. 1997; Sangha *et al*. 1998) and other species (Menasveta *et al*. 1994; Cavalli *et al*. 1997). The only viable way to obtain sufficient research data in these conditions was to stock and monitor a large number of animals in the maturation tanks.

On average, 84% of the feeding regime in commercial facilities of the western hemisphere is based on fresh food items like squid, mussel, marine Polychaetes and others, while in Asia a total dependence on fresh food seems to be the rule (Wouters *et al.* 2000). The reason for this is that high replacement levels of fresh food with artificial diets reduce broodstock performance and offspring quality (Harrison 1990; Bray *et al.* 1990; Bray & Lawrence 1992), because artificial diets do not meet the exact nutritional requirements of shrimp during the maturation process and because of the preferential ingestion of fresh food over artificial diet. The present study clearly suggests the feasibility of substituting 50% of the fresh food with artificial broodstock diets. This fresh food substitution did not affect the response variables negatively. On the contrary, spawn frequency doubled in experiment 1 (treatments CON and ART) and the production of eggs per female doubled in experiment 2 (treatment ART). In both experiments, repeat spawn performance was improved in the treatments fed the artificial diets, which means that spawner exhaustion – that typically occurs over time in captive broodstock animals – was overcome. Female performance was better in treatments receiving artificial diets than in the fresh food treatment, thus supporting the quality of our practical artificial diet. The development of the diet used in this study was based upon the information acquired from literature and analysis of existing commercial broodstock diets (Wouters *et al.* 2001), and a series of trial-and-error experiments. The present results confirm the findings of Nascimento *et al.* (1991) with *Litopenaeus schmitti*, that combining fresh food and artificial diets in a shrimp broodstock feeding regime is a better practice than relying on fresh food alone. Several abstracts have been published from studies that also evaluated a 50% fresh food substitution with dry diets: Verstraete *et al.* (1995) and Coutteau *et al.* (1998) with *L. vannamei*, and Denece *et al.* (1999) with *Litopenaeus stylirostris*. Verstraete *et al.* (1995) obtained an increased maturation frequency in tanks fed the dry diet, but a decrease in egg hatching percentage and spawning size. At the end, the production of nauplii per female remained unaffected by the partial fresh food replacement. Coutteau *et al.* (1998) obtained an improved survival rate, but an inferior production of nauplii per female. Denece *et al.* (1999) obtained similar reproductive performance and nauplii quality with dry diets (50%) or fresh food. None of the previous studies reported results as positive as those obtained in the present study.

Fresh food substitution levels above 50% have not yet proven successful. In contrast, moist artificial diets or artificial diets containing minced fresh food have been used successfully for total fresh food substitution by Galgani *et al.* (1989) and Galgani & AQUACOP (1989) for *L. stylirostris*, *L. vannamei* and *Fenneropenaeus indicus*, and by Marsden *et al.* (1997) with *Penaeus monodon*. However, these diets have a short shelf-life and low water stability, limiting commercial application.

It is worth emphasizing the fact that the fresh food items that were substituted in the present commercial hatchery trial were precisely those that are presumed indispensable for normal maturation and reproduction, i.e. bloodworms and enriched *Artemia* biomass (Browdy 1992; Kawahigashi 1992; Naessens *et al.* 1997). In hatcheries of the western hemisphere, bloodworms constitute the most expensive broodstock diet ingredient (Rhodes *et al.* 1992; Kawahigashi 1992, 1998), and their replacement with dry diet is set as a priority by various authors (Harrison 1997).

In a preliminary study (Wouters 2001), it was found that incorporating freeze-dried *Artemia* biomass into an artificial broodstock diet increased diet ingestion, improved gonad maturation in female and male *L. vannamei*, and increased spawning and repeat spawning performance. This beneficial effect of *Artemia* meal was, however, more pronounced in pond reproducers than in wild reproducers. The present study looks further into the potential of *Artemia* meal as an ingredient in dry diets for wild reproducers. In experiment 1, a positive effect of *Artemia* meal inclusion could not be confirmed. On the contrary, in experiment 2 the inclusion of freeze-dried *Artemia* to the artificial diet had various positive effects: more spawns, better repeat spawn performance, higher egg production per female, higher sperm counts and an increased spermatophore weight. These responses suggest that the beneficial effects of fresh-frozen *Artemia* biomass, as reported in Wouters *et al.* (1999) for the same reproductive parameters, are maintained after processing it into a meal and including it to an artificial diet.

The results of experiment 1 were sometimes different to those obtained in experiment 2 (the effect of fresh food substitution, the effect of *Artemia* meal inclusion, sperm counts, offspring quality). It is impossible to pinpoint one cause for these observed differences. Seasonal-environmental effects and geographical effects may have contributed to these differences (Hansford & Marsden 1995; Marsden *et al.* 1997). Different maturation facilities and environmental conditions might also have contributed to the observed variation (Bray & Lawrence 1992).

In conclusion, this study suggests the feasibility of substituting 50% of the classical fresh food regime with practical dry artificial diets in *L. vannamei* broodstock facilities. In this way, bloodworms and *Artemia* biomass may be deleted from the feeding regime. The use of *Artemia* meal appears to further improve the performance of the artificial diets.

Acknowledgements

Our sincere gratitude goes to David Garriques and Eloy Calderón of Granjas Marinas (El Rosario S.A) for allowing us to conduct research in their facilities and for providing invaluable assistance and advise. This work was supported by the VLIR-Own Initiative Program of the Flemish Inter-University Council (VL.I.R). At the time of this study, Byron Zambrano was a student of the Universidad Técnica de Manabi (Ecuador) and Marcos Espín was a student of the Escuela Superior Politécnica del Litoral (Ecuador).

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