Aquaculture Nutrition

Aquaculture Nutrition **2011 17;** e511–e520 doi: 10.1111/j.1365-2095.2010.00791.x

Growth performance of the white shrimp, *Litopenaeus vannamei*, fed on practical diets with increasing levels of the Antarctic krill meal, *Euphausia superba*, reared in clear- versus green-water culture tanks

A.J.P. NUNES, M.V.C. SÁ & H. SABRY-NETO

LABOMAR – Instituto de Ciências do Mar, Universidade Federal do Ceará, Avenida da Abolição, Fortaleza, Ceará, Brazil

Abstract

Litopenaeus vannamei were stocked in 25 clear-water 500-L tanks at 100 shrimp m^{-2} and in 25 green-water 1000-L tanks at 60 animals m^{-2}. Four diets were formulated to include krill meal at 10, 50 or 110 g kg^{-1}; or krill oil at 25 g kg^{-1} by replacing fish meal, fish oil, soybean lecithin and cholesterol. Diets had similar levels of crude protein, total energy and essential amino acids. After 72 days, shrimp reared in clear and green water showed no differences in performance among diets. In clear water, shrimp attained 13.1 ± 0.59 g body weight, 1.00 ± 0.06 g $week^{-1}$ growth, 81.4 ± 7.3% survival, 780 ± 118 g m^{-2} yield, 16.9 ± 1.8 g $shrimp^{-1}$ apparent feed intake (AFI), and 2.18 ± 0.29 food conversion ratio (FCR). In green water, shrimp attained 14.3 ± 0.81 g body weight, 1.04 ± 0.09 g $week^{-1}$ growth, 91.4 ± 5.4% survival, 569 ± 69 g m^{-2} yield, 20.9 ± 3.2 g $shrimp^{-1}$ AFI, and 2.22 ± 0.34 FCR. Diets containing krill meal or krill oil were able to fully replace the protein and lipid value of fish meal, fish oil, soybean lecithin and cholesterol at no cost to performance.

KEY WORDS: fish meal, Krill meal, Krill oil, *Litopenaeus vannamei*, shrimp

Received 15 July 2009, accepted 9 April 2010

Correspondence: Alberto Jorge Pinto Nunes, LABOMAR – Instituto de Ciências do Mar, Universidade Federal do Ceará, Avenida da Abolição, 3207 – Meireles, Fortaleza, Ceará, 60.165-081, Brazil. E-mail: albertojpn@uol.com.br

Introduction

Diets manufactured for the rearing of penaeid shrimp have been based on fish meal and fish oil as their major sources of protein and lipid. However, small pelagic marine fish stocks that are caught to produce fish meal and fish oil are fully exploited, and no significant increase in production is forecast to occur (FAO 2009). Thus, there is an ongoing effort to find suitable substitutes or alternatives for fish meal and fish oil for use in aquaculture feeds.

Several plant and land animal protein sources have been tested so far, but none have been able to fully replace the individual value of fish meal or fish oil. Plant protein by-products, such as soybean, canola, pea and sunflower meal, have deficiencies in some essential amino acids (Gatlin *et al.* 2007) and also contain anti-nutritional factors (Francis *et al.* 2001). On the other hand, rendered animal protein sources of terrestrial origin, such as ruminant meat and bone meal, and blood meal have poor attractability properties for penaeid shrimp (Nunes *et al.* 2006). Therefore, the search for raw materials nutritionally analogous or similar to fish meal is still continuing.

Among the current options under investigation, krill meal and krill oil are promising candidates for regular application in aquaculture feeds. The Antarctic krill, *Euphausia superba*, has a large standing biomass (Nicol & Foster 2003), estimated at 44.3 million tons in Antarctica's South Atlantic sea (CCAMLR 2000; Trathan *et al.* 2001; Watkins *et al.* 2004). However, only a modest increase in krill catches is expected to occur in the future because of stringent conservation measures and high costs of fisheries in the cold waters of Antarctica.

Studies on the use of krill meal in fish diets began in the 1970s. The general conclusion of these studies was that krill is a valuable nutritional source to farmed fish, but maximum inclusion level in the diet is restricted by its chitin, copper and, mainly, fluoride concentration (Olsen *et al.* 2006).

Although some consensus exists for the use of krill meal in fish diets, scarce information is available regarding its use in

penaeid shrimp diets. Studies on the use of krill meal in shrimp feeds have focused on its use as a feed supplement to enhance growth or to promote feed intake (Córdova-Murueta & García-Carreño 2002; Smith *et al*. 2005; Williams *et al*. 2005). No work exists on the use of krill meal or krill oil as ingredient sources in diets for juvenile *Litopenaeus vannamei*. Therefore, the present work aimed at investigating the growth performance of juvenile *L. vannamei* fed on practical diets with increasing levels of Antarctic krill meal or krill oil and decreasing levels of fish meal, fish oil, cholesterol and soybean lecithin. The study was conducted simultaneously in clear-water and green-water culture systems.

Material and methods

Shrimp and culture system

The study was conducted in the indoor and outdoor tank facilities of the Laboratory of Aquatic Animal Nutrition (3°50′01.55″S and 38°25′22.74″W) located in Eusébio, Brazil. The laboratory is part of the Instituto de Ciências do Mar (LABOMAR) of the Federal University of the State of Ceará (Fortaleza, Brazil).

Juvenile shrimp (1.93 ± 0.56 g; mean ± standard deviation; $n = 100$) of *L. vannamei* were collected from one commercial grow-out pond of the Le Crevette farm, located 18 km away from the experimental facilities. A total of 12 000 juveniles were transported in plastic bags containing seawater and pure oxygen (120 shrimp for every 20 L of water).

Immediately after transportation, shrimp were individually counted and stocked in rearing tanks. Shrimp originated from the same farm post-larvae batch and were stocked over two consecutive days, first in indoor tanks at 57 shrimp $tank^{-1}$ (100 shrimp m^{-2} bottom surface area or 114 shrimp m^{-3}), and in the next day, in outdoor tanks at 61 shrimp $tank^{-1}$ (60 animals m^{-2} bottom surface area or 61 shrimp m^{-3}). The use of different stocking densities in each particular system relate to water quality and natural food availability.

A total of 25 outdoor and 25 indoor tanks were assigned to this trial. Outdoor and indoor tanks are round with a total volume and bottom surface area of 1000 and 500 L and 1.02 and 0.57 m^2, respectively. The outdoor system operated under green-water conditions and was continually exposed to weather changes. Comparatively, indoor tanks operated under clear-water conditions (no or poor availability of natural food items). They were sheltered from significant weather changes, and water was continually sand filtered overnight for removal of solid wastes. Under indoor conditions, shrimp were kept under a 12-h artificial light cycle during the complete study period. Both systems were provided with constant aeration supplied from mechanical blowers.

Feed design and preparation

The present study evaluated four treatment feeds containing either krill meal or krill oil and a basal (control) diet deprived of these ingredients (Table 1). Five replicate tanks were designated for each treatment and basal diet, which were randomly assigned in both the clear- and green-water systems.

The basal diet was formulated to contain levels of fish meal (187.5 g kg^{-1}), fish oil (20 g kg^{-1}), soybean lecithin (15 g kg^{-1}), and cholesterol (1.5 g kg^{-1}) to fully meet penaeid shrimp nutritional requirements (Akiyama *et al*. 1991; Glencross *et al*. 2002). No addition of krill meal or krill oil was made in the basal diet. The basal and the experimental diets had similar levels of crude protein (CP), total energy and essential amino acids (Tables 1 & 2). As the chitin content of crustacean meals cannot be used as a source of amino acids to synthesize the animal's body proteins (Akiyama *et al*. 1991), the nitrogen content of krill meal's chitin was deducted from the CP calculations following the data presented by Olsen *et al*. (2006). The experimental diets were formulated to include krill meal at 10 g kg^{-1} (KM10), 50 g kg^{-1} (KM50) or 110 g kg^{-1} (KM110); or krill oil at 25 g kg^{-1} (KO25), by replacing fish meal, fish oil, broken rice, soybean lecithin and purified cholesterol. Replacement of these ingredients was carried out on the basis of CP, total lipid, essential amino acid, cholesterol, and phospholipids to take full advantage of the krill meal and krill oil nutritional value.

Diets KM10, KM50 and KM110 progressively reduced the contribution of fish meal to total feed protein input in comparison with the basal diet while increasing inclusion level of krill meal. Concomitantly, level of meat and bone meal and soybean meal were raised to meet targeted feed protein levels. As a result, KM10, KM50 and KM110 contained a lower amount of marine animal protein sources and increased levels of land animal and vegetable protein sources. KM10, KM50 and KM110 also progressively reduced the inclusion of fish oil, soybean lecithin and cholesterol as higher levels of krill meal were used.

In diet KO25, krill oil was used to fully replace cholesterol and to partially replace fish oil and soybean lecithin. In this case, all other macro ingredients kept the same inclusion level as the basal diet, except in regard to meat and bone meal which was reduced to compensate higher lipid levels as a result of krill oil addition.

Diets were manufactured with laboratory-scale feed equipment. Initially, all grain ingredients (except wheat flour)

Table 1 Ingredient composition and proximate chemical analysis of the experimental diets (g kg^{-1} as is)

	Experimental diet composition				
Ingredients	Basal	KO25	KM10	KM50	KM110
Soybean meal [1]	300.0	300.5	340.2	338.6	349.4
Wheat flour [2]	250.0	250.0	250.0	250.0	250.0
Fish meal, Anchovy [3]	150.0	150.0	100.0	50.0	0.0
Broken rice [4]	130.4	134.5	115.6	108.0	113.5
Fish meal, by-catch [5]	37.5	37.5	25.0	12.5	0.0
Corn gluten meal [6]	30.0	30.0	30.0	30.0	30.0
Meat and bone meal [7]	23.1	17.0	55.0	100.0	99.5
Fish oil [8]	20.0	8.0	15.2	17.7	0.1
Soybean lecithin [9]	15.0	5.0	15.0	0.0	0.0
Krill meal [10]	0.0	0.0	10.0	50.0	115.0
Krill oil [11]	0.0	25.0	0.0	0.0	0.0
Cholesterol [12]	1.5	0.0	1.5	0.8	0.0
Magnesium sulfate	1.5	1.5	1.5	1.5	1.5
Potassium chloride	4.0	4.0	4.0	4.0	4.0
Salt, common	10.0	10.0	10.0	10.0	10.0
Vitamin–mineral premix [13]	10.0	10.0	10.0	10.0	10.0
Synthetic Binder [14]	5.0	5.0	5.0	5.0	5.0
Bicalcium phosphate	12.0	12.0	12.0	12.0	12.0
Proximate composition (g kg^{-1} DM) [15]					
CP	325.5	332.5	336.0	327.3	338.6
Total lipids	103.8	100.8	103.5	107.5	107.5
Ash	99.5	93.5	98.3	99.5	96.7
Crude fiber	1.65	1.31	1.49	1.64	2.22
Gross energy (kJ g^{-1})	17.5	16.2	17.5	16.9	16.8
Moisture (% as fed)	11.1	8.2	10.7	10.2	8.8
Selected mineral composition (mg kg^{-1})					
Fluoride	0	0	15	75	165

[1] Bunge Alimentos S.A. (Luis Eduardo Magalhães, Brazil). 462.0 g kg^{-1} crude protein (CP); 21.0 g kg^{-1} total lipid (TL); 101.2 g kg^{-1} ash; 77.4 g kg^{-1} crude fat (CF); 19.7 kJ g^{-1} gross energy; values at dry matter basis (DM).

[2] Moinho Dias Branco Ltda. (Fortaleza, Brazil). 130.4 g kg^{-1} CP; 12.3 g kg^{-1} TL; 7.8 g kg^{-1} ash; 0.6 g kg^{-1} CF; 18.0 kJ g^{-1} GE; values at DM.

[3] COPEINCA Corporación Pesquera INCA S.A. (Lima, Peru). 688.7 g kg^{-1} CP; 69.8 g kg^{-1} fat; 162.8 g kg^{-1} ash; 1.2 g kg^{-1} TL; 16.7 kJ g^{-1} GE; values at DM.

[4] Usina Catende (Catende, Brazil). 81. 8 g kg^{-1} CP; 17. 5 g kg^{-1} TL; 8.8 g kg^{-1} ash; 1.1 g kg^{-1} CF; 14.8 kJ g^{-1} GE; values at DM.

[5] INPEL Indústria de Resíduos de Pescado Ltda. (Canoas, Brazil). 565.5 g kg^{-1} CP; 57.2 g kg^{-1} TL; 233.3 g kg^{-1} ash; 8.1 g kg^{-1} CF; 14.3 kJ g^{-1} GE; values at DM.

[6] Protenose®, Corn Products Brasil (São Paulo, Brazil). 625.3 g kg^{-1} CP; 95.1 g kg^{-1} TL; 46. 6 g kg^{-1} ash; 2.1 g kg^{-1} CF; 20.9 kJ g^{-1} GE; values at DM.

[7] NORDAL Nordeste Ind. de Derivados Animais Ltda. (Maracanaú, Brazil). 462.9 g kg^{-1} CP; 164. 6 g kg^{-1} TL; 311.3 g kg^{-1} ash; 6.4 g kg^{-1} CF; 18.5 kJ g^{-1} GE; values at DM.

[8] COPEINCA Corporación Pesquera INCA S.A. (Lima, Peru). 980.0 g kg^{-1} TL; 38.7 kJ g^{-1} GE.

[9] Cargill Nutrição Animal Ltda. (São Paulo, Brazil). 1000.0 g kg^{-1} TL; 31.7 kJ g^{-1} GE.

[10] QRILL™, Aker Biomarine ASA (Oslo, Norway). 590.0 g kg^{-1} CP; 250.0 g kg^{-1} TL; 100.0 g kg^{-1} ash; 0.0 g kg^{-1} CF; 18.6 kJ g^{-1} GE; values at DM.

[11] QRILL™, Aker Biomarine ASA (Oslo, Norway). 980.0 g kg^{-1} fat; 38.7 kJ g^{-1}.

[12] Cholesterol XG, Solvay Pharmaceuticals BV (Weesp, the Netherlands). 910.0 g kg^{-1} cholesterol.

[13] Rovimix Camarao Intensivo, DSM Produtos Nutricionais Brasil Ltda. (São Paulo, Brazil). Guarantee levels per kg of product: vitamin A, 1 250 000 IU; vitamin D3, 350 000 IU; vitamin E, 25 000 UI; vitamin K3, 500.0 mg; vitamin B1, 5000.0 mg; vitamin B2, 4000.0 mg; vitamin B6; 10.0 mg; nicotinic acid, 15 000.0 mg; pantothenic acid, 10 000.0 mg; biotin, 150.0 mg; folic acid, 1250.0 mg; vitamin C, 25 000.0 mg; cholin, 50 000.0 mg; inositol, 20 000.0 mg; iron 2000.0 mg; copper, 3500.0 mg; chelate copper, 1500.0 mg; zinc, 10 500.0 mg; chelate zinc, 4500.0 mg; manganese, 4000.0 mg; selenium, 15.0 mg; chelate selenium, 15.0 mg; iodine, 150.0 mg; cobalt, 30.0 mg; chromium 80.0 mg; filler, 1000.0 g.

[14] Pegabind™; Bentoli Agrinutrition (Elgin, TX, USA). Synthetic pellet binder composed of urea formaldehyde.

[15] Reported as dry matter basis; values are means of two replicate analyses; dietary fluoride calculated from formulated values.

Table 2 Amino acid profile of the experimental diets (g kg^{-1} DM; analysed values)

Amino acid	Amino acid profile (g 100 g^{-1} diet)				
	Basal [1]	KO25 [2]	KM10 [3]	KM50 [4]	KM110 [5]
Alanine	15.8	15.7	16.7	15.1	16.6
Arginine	17.8	18.1	19.8	18.3	20.3
Aspartic acid	35.7	30.9	31.0	33.4	36.4
Glycine	17.3	17.3	19.6	18.2	19.6
Isoleucine	12.7	12.0	13.7	12.4	13.7
Leucine	24.3	23.4	25.6	23.4	25.6
Glutamic acid	63.7	60.6	64.2	61.1	67.9
Lysine	17.0	16.6	17.8	15.3	16.7
Cystine	2.4	2.5	1.8	2.1	3.7
Methionine	10.3	10.0	9.0	8.7	9.8
Phenylalanine	13.8	12.9	14.5	13.3	14.9
Tyrosine	8.9	8.7	9.3	8.4	9.7
Threonine	11.6	11.2	11.9	10.7	11.9
Tryptophan	2.4	2.3	2.5	2.1	2.3
Proline	9.5	9.3	10.3	9.6	10.6
Valine	13.6	12.9	14.4	13.0	14.5
Histidine	5.0	4.9	5.0	4.7	5.4
Serine	14.5	14.5	15.4	13.7	15.5

[1] Basal: control diet with regular levels of wheat flour (250 g kg^{-1}), soybean meal (300 g kg^{-1}), fish meal (18.75 g kg^{-1}; 80% Anchovy fish meal + 20% by-catch fish meal), fish oil (20 g kg^{-1}), soybean lecithin (15 g kg^{-1}) and cholesterol (1.5 g kg^{-1}); no inclusions of krill meal or krill oil.

[2] KO25: basal diet with 60% reduction in fish oil (20–8 g kg^{-1}), 2/3 reduction in soybean lecithin (15–5 g kg^{-1}), no cholesterol and inclusion of krill oil at 25 g kg^{-1}.

[3] KM10: basal diet with 1/3 reduction in fish meal (187.5–125.0 g kg^{-1}), inclusion of krill meal at 10 g kg^{-1} and 20% reduction in fish oil (20–16 g kg^{-1}).

[4] KM50: basal diet with 2/3 reduction in fish meal (187.5–62.5 g kg^{-1}), inclusion of krill meal at 50 g kg^{-1}, regular levels of fish oil (20 g kg^{-1}), no soybean lecithin and a nearly 50% reduction in cholesterol (1.5–0.8 g kg^{-1}).

[5] KM110: basal diet without fish meal, inclusion of krill meal at 110 g kg^{-1}, 60% reduction in fish oil (20–8 g kg^{-1}), no soybean lecithin and no cholesterol.

were ground in a coffee-grinder to achieve a particle size lower than 400 microns. Marine animal by-products were sieved through a 225-micron mesh net. After grinding or sieving, all dry and liquid ingredients were weighed to a 0.01 electronic scale and mechanically mixed for 15 min. Boiling water was added to the ingredient mixture at a rate of 2 : 1 and allowed to mix for 20 min until a feed dough was produced. The feed dough was then transferred to a pot for 20-min steam-cooking to reach an internal temperature of 95 °C in the feed dough. After steam-cooking, the feed cake was immediately moved to a meat mincer for double extrusion of feed particles to a 2-mm diameter. All feed pellets in a spaghetti-like format were dried in a convection oven at 70 °C. Feed was turned over twice every 2 h prior to disintegration of pellets to a 5-mm length with a food chopper. After chopping of pellets, an additional 1 h of drying was allowed to reach a final feed moisture content of 8–10%. Finished diets were stored in air-tight containers at −20 °C until use.

Management and feeding

Prior to the start of the growth study, shrimp were first reared for 10 days on a commercial extruded diet (Aquaxcel™; Burris Mill Aquaculture, Franklinton, LA, USA). Feed contained 420 g kg^{-1} of CP and 90 g kg^{-1} of fat. After the acclimation period, shrimp started to be fed on laboratory-manufactured diets. Shrimp were continuously exposed to diets over 24-h periods. To avoid feed loss in tanks and rapid water quality deterioration, all feed was delivered in feeding trays made from a rectangular nylon mesh of 141 mm in diameter surrounded by a PVC circular frame of 150 mm in diameter and 3.5 cm in height. Trays were installed in the middle of each tank bottom at a density of one unit per tank.

Animals were fed twice daily at 0730 h and 1600 h over the complete rearing period. Meals were delivered on a feed consumption basis, allowing rough changes in feed rations to take place at each feeding time. When necessary, adjustments in meals were carried out in respect to each feeding time (i.e. feed remains at 0700 h to adjust feed rations for 0730 h the next day). A protocol was used to adjust rations 25% above or below original calculated meals. On Sundays, shrimp were fed only in the morning with 100% of the daily meal. In this case, uneaten feed was collected the next day after a 24-h immersion period.

Water pH, temperature and salinity were monitored once daily in the morning in each tank. In the clear-water system, sand filters only operated overnight from 1700 h to 0700 h in the morning. Whenever filter pressure increased, tank water was back flushed to discharge organic solid material. Back flushing took place within an interval of 7–15 days or depending on shrimp stage of development. In the green-water system, 25% of culture water was discharged on a weekly basis from each tank and replaced by newly pumped brackish water.

Shrimp were reared for 72 days in both the clear- and green-water tanks. On a 3.5-weekly basis, 10 animals of each tank were captured to determine their individual wet body weight in an electronic scale. After weighing, all animals were returned to their respective rearing tank. No sampling was carried out 3 days before or after the full and new moon. Over these periods, shrimp are less active in feeding and more prone to shedding of their exoskeleton.

At harvest, shrimp were weighed and counted to determine their final wet body weight (g), weekly growth rate

(g week^{-1}), final survival (%), yield (g m^{-2}), apparent feed intake (AFI, g shrimp^{-1}), and food conversion ratio (FCR). AFI was calculated by subtracting feed leaching and water absorption rates from the amount of feed delivered (on the dry basis) over the growth cycle (Nunes *et al*. 2006). The sum of feed intake per tank was then divided by the number of stocked shrimp in each rearing unit. FCR was determined on the basis of AFI.

Chemical analyses

Diets were analysed for proximate composition (Table 1) and amino acid profile (Table 2) using standard reference methods (AOAC 2002). Briefly, moisture was determined gravimetrically after drying at 105 °C for 24 h. Ash was determined by incineration at 550 °C in a muffle oven. Total nitrogen was determined by distillation according to the Kjeldahl method. CP was calculated as N × 6.25. Lipid was determined gravimetrically after ether extraction. Crude fibre was determined by acid and alkaline hydrolysis. Gross energy was determined by the complete combustion of samples with a Parr calorimeter. The fluorine content of the diets was calculated from the ingredient compositions and the tabulated values presented by Hertramph & Piedad-Pascual (2000). Amino acid analysis was carried out by high-performance liquid chromatography.

Statistical analyses

Shrimp biological performance data fed the different diets were subjected to one-way analysis of variance (ANOVA) for completely randomized experiments. Survival data were arcsine-transformed before analysis. The Student's *t*-test was used to compare differences between the two rearing systems (clear or green water). The data was checked for normality and all analyses were performed with the SPSS 15.0 software package (SPSS Inc., Chicago, IL, USA).

Results

Water quality

Water quality parameters remained within the ideal range for the culture of penaeid shrimp. No statistical differences were observed for water quality between feeding treatments ($P > 0.05$, ANOVA), regardless of the rearing system adopted. In the clear-water system, water salinity, pH and temperature averaged 32.4 ± 2.6 ppt ($n = 1225$), 7.72 ± 0.23 ($n = 1225$) and 27.4 ± 0.8 °C ($n = 1225$), respectively. Over the rearing period, there was an increase in water salinity, consistent with the dry season that takes place in the experimental site from mid June until January (Fig. 1). During these months, there is often an increase in salinity in the water source used in shrimp nutrition studies. Water temperature remained consistent throughout the study period, while water pH dropped.

In the green-water system, water salinity, pH and temperature averaged 36.6 ± 3.5 ppt ($n = 1150$), 8.13 ± 0.17 ($n = 1150$) and 29.6 ± 0.9 °C ($n = 1150$), respectively. As opposed to the clear-water system, drops in water pH in green-water tanks were less intense. On the other hand, water salinity peaked at 40 ppt in comparison to 35 ppt in the clear-water system. On average, water temperature in green-water tanks was 2.2 °C higher than in clear-water tanks ($P < 0.05$, *t*-test).

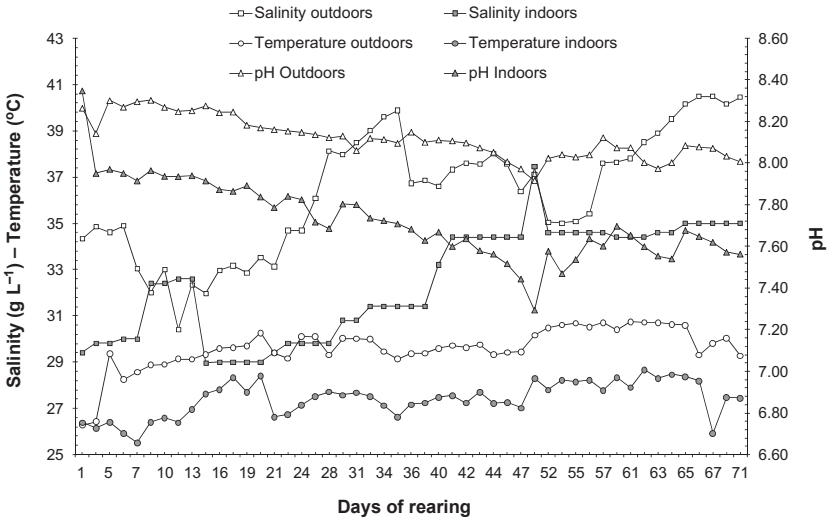

Figure 1 Daily water quality fluctuation in salinity (g L^{-1}), temperature (°C) and pH over a 72-day the rearing cycle of *Litopenaeus vannamei* farmed under a clear-water (indoor) and green-water (outdoor) culture system. Each data point refers to the daily average values of five rearing tanks.

Shrimp performance

Shrimp grew continuously over the culture period regardless of the rearing system adopted (Table 3). Over the growth cycle, statistical differences in shrimp wet body weight could not be observed between experimental diets when shrimp were farmed under a clear- or a green-water condition ($P > 0.05$, ANOVA). In clear-water tanks, gains in wet body weight were very consistent for all tested diets. Conversely, in green water, shrimp fed the basal and KO25 diets achieved a higher final body weight than those fed diets KM10, KM50 and KM110, but differences were not statistically significant ($P > 0.05$, ANOVA).

Under both rearing systems, shrimp weekly growth rates did not differ statistically between diets ($P > 0.05$; ANOVA; Table 4). The average weight gain ranged from 0.98 to 1.01 g week^{-1} and 1.01 to 1.08 g week^{-1} for the clear- and green-water systems, respectively. While under green-water conditions, shrimp consistently kept individual growth rates above 1.00 g week^{-1} over the growth cycle, in clear-water tanks they remained below or close to 1.00 g week^{-1} regardless of diet type. Comparatively, a higher shrimp growth was achieved in green-water than in clear-water conditions ($P < 0.05$, *t*-test; Table 4).

At harvest, final shrimp survival rate was above 80% in all feeding treatments. Shrimp yield and final survival showed no statistical differences between experimental diets ($P > 0.05$, ANOVA; Table 4), but differed between the two rearing systems ($P < 0.05$, *t*-test; Table 4). As a result of a more intensive rearing condition for shrimp reared indoors, a 37% higher yield was achieved in clear-water than under green-water conditions ($P < 0.05$, *t*-test; Table 4). This occurred despite a lower final shrimp survival obtained in clear water versus in green water (81.4 ± 7.3% versus 91.4 ± 5.4%, respectively; $P < 0.05$, *t*-test).

In clear-water tanks, AFI tended to be slightly higher for shrimp fed diets containing krill meal or krill oil (KO25, KM10, KM50 and KM110) than for the basal diet, but differences were not statistically significant ($P > 0.05$, ANOVA; Table 4). In green-water tanks, there was no clear trend or statistical differences in AFI for the various feed types ($P > 0.05$, ANOVA; Table 4). AFI was 24% higher for shrimp stocked in green water compared to clear water ($P < 0.05$, *t*-test; Table 4). Finally, the FCR varied from 1.9 to <2.5 (Table 4). Contrary to other performance parameters, FCR showed no differences in regard to diet type ($P > 0.05$, ANOVA) and rearing system ($P > 0.05$, *t*-test, Table 4).

Table 3 Gains in *Litopenaeus vannamei* wet body weight (in grams) reared in two-culture systems fed diets containing either krill meal or krill oil in full or partial replacement of fish meal, fish oil, soybean lecithin and/or cholesterol. Shrimp were stocked at 100 and 60 shrimp m^{-2} in 25 clear and green-water tanks, respectively. Each value refers to the mean ± standard deviation obtained from five rearing tanks. A total of 10 shrimp were sampled per tank, except in day 72 which included all harvested shrimp

		Days of rearing			
Culture system	Diet [1]	1	26	48	72
Clear water	Basal	2.83 ± 0.22	6.21 ± 1.25	9.72 ± 0.85	13.03 ± 0.77
	KO25	2.94 ± 0.28	6.12 ± 0.78	9.51 ± 0.46	13.03 ± 0.24
	KM10	2.93 ± 0.19	6.25 ± 0.55	9.40 ± 1.03	13.29 ± 0.60
	KM50	2.82 ± 0.25	6.12 ± 0.45	9.53 ± 0.57	12.91 ± 0.55
	KM110	2.97 ± 0.17	5.95 ± 0.38	9.79 ± 0.66	13.37 ± 0.79
ANOVA *P*		ns[2]	ns	ns	ns
Green water	Basal	3.52 ± 0.17	7.74 ± 0.41	11.04 ± 0.73	14.68 ± 0.54
	KO25	3.76 ± 0.05	7.75 ± 0.18	11.63 ± 0.60	14.63 ± 0.78
	KM10	3.53 ± 0.28	7.55 ± 0.38	10.50 ± 0.68	13.95 ± 0.53
	KM50	3.52 ± 0.24	7.78 ± 0.84	10.75 ± 1.14	14.00 ± 1.34
	KM110	3.41 ± 0.31	7.67 ± 0.59	11.10 ± 0.62	14.19 ± 0.56
ANOVA *P*		ns	ns	ns	ns

[1] Basal: control diet with regular levels of wheat flour (250 g kg^{-1}), soybean meal (300 g kg^{-1}), fish meal (18.75 g kg^{-1}; 80% Anchovy fish meal + 20% by-catch fish meal), fish oil (20 g kg^{-1}), soybean lecithin (15 g kg^{-1}) and cholesterol (1.5 g kg^{-1}); no inclusions of krill meal or krill oil; KO25: basal diet with 60% reduction in fish oil (20–8 g kg^{-1}), 2/3 reduction in soybean lecithin (15–5 g kg^{-1}), no cholesterol and inclusion of krill oil at 25 g kg^{-1}; KM10: basal diet with 1/3 reduction in fish meal (187.5–125.0 g kg^{-1}), inclusion of krill meal at 10 g kg^{-1} and 20% reduction in fish oil (20–16 g kg^{-1}); KM50: basal diet with 2/3 reduction in fish meal (187.5–62.5 g kg^{-1}), inclusion of krill meal at 50 g kg^{-1}, regular levels of fish oil (20 g kg^{-1}), no soybean lecithin and a nearly 50% reduction in cholesterol (1.5–0.8 g kg^{-1}); KM110: basal diet without fish meal, inclusion of krill meal at 110 g kg^{-1}, 60% reduction in fish oil (20–8 g kg^{-1}), no soybean lecithin and no cholesterol.

[2] Not significant ($P > 0.05$).

Table 4 Growth performance (mean ± standard deviation) of *Litopenaeus vannamei* juveniles reared for 72 days in 500-L indoor (clear water) and 1000-L outdoor (green water) polyethylene tanks. Shrimp were fed with isoprotein (330 g kg^{-1}) and isolipidic (100 g kg^{-1}) diets containing different levels of krill meal or krill oil in replacement of fish meal, fish oil, cholesterol, and/or soybean lecithin. Means refer to a total of five tanks. In each culture system, the differences between means for all parameters were not significant between different diets ($P > 0.05$; ANOVA). The last column refers to comparisons between culture systems

Performance	Culture system	Experimental diet [1]						*P* Sig. (Student's *t*-test)
		Basal	KO25	KM10	KM50	KM110	Mean ± SD	
Growth (g $week^{-1}$)	Indoor	0.98 ± 0.02	0.99 ± 0.06	1.01 ± 0.07	0.98 ± 0.07	1.01 ± 0.08	1.00 ± 0.06	<0.05
	Outdoor	1.06 ± 0.08	1.08 ± 0.05	1.01 ± 0.08	1.02 ± 0.14	1.05 ± 0.07	1.04 ± 0.09	
Final shrimp survival (%)	Indoor	83.9 ± 10.1	83.5 ± 10.7	80.0 ± 6.2	81.4 ± 6.9	80.7 ± 6.0	81.4 ± 7.3	<0.05
	Outdoor	91.8 ± 4.6	93.4 ± 2.6	94.4 ± 1.9	91.5 ± 3.6	85.9 ± 8.7	91.4 ± 5.4	
Shrimp yield (g m^{-2})	Indoor	801 ± 130	808 ± 169	772 ± 144	770 ± 125.5	778 ± 40	780 ± 118	<0.05
	Outdoor	578 ± 48	610 ± 51	576 ± 35	555 ± 94	525 ± 92	569 ± 69	
AFI[2] (g $shrimp^{-1}$)	Indoor	15.8 ± 1.8	17.8 ± 1.0	16.5 ± 2.1	16.6 ± 2.2	17.6 ± 1.4	16.9 ± 1.8	<0.05
	Outdoor	21.4 ± 3.8	23.9 ± 4.4	18.9 ± 0.4	19.6 ± 2.0	20.8 ± 2.4	20.9 ± 3.2	
FCR[2]	Indoor	1.99 ± 0.25	2.26 ± 0.29	2.18 ± 0.37	2.18 ± 0.33	2.27 ± 0.18	2.18 ± 0.29	ns[3]
	Outdoor	2.47 ± 0.26	2.08 ± 0.21	1.96 ± 0.10	2.13 ± 0.18	2.44 ± 0.54	2.22 ± 0.34	

[1] Basal: control diet with regular levels of wheat flour (250 g kg^{-1}), soybean meal (300 g kg^{-1}), fish meal (18.75 g kg^{-1}; 80% Anchovy fish meal + 20% by-catch fish meal), fish oil (20 g kg^{-1}), soybean lecithin (15 g kg^{-1}) and cholesterol (1.5 g kg^{-1}); no inclusions of krill meal or krill oil; KO25: basal diet with 60% reduction in fish oil (20–8 g kg^{-1}), 2/3 reduction in soybean lecithin (15–5 g kg^{-1}), no cholesterol and inclusion of krill oil at 25 g kg^{-1}; KM10: basal diet with 1/3 reduction in fish meal (187.5–125.0 g kg^{-1}), inclusion of krill meal at 10 g kg^{-1} and 20% reduction in fish oil (20–16 g kg^{-1}); KM50: basal diet with 2/3 reduction in fish meal (187.5–62.5 g kg^{-1}), inclusion of krill meal at 50 g kg^{-1}, regular levels of fish oil (20 g kg^{-1}), no soybean lecithin and a nearly 50% reduction in cholesterol (1.5–0.8 g kg^{-1}); KM110: basal diet without fish meal, inclusion of krill meal at 110 g kg^{-1}, 60% reduction in fish oil (20–8 g kg^{-1}), no soybean lecithin and no cholesterol.
[2] AFI, apparent feed intake per stocked shrimp (g $shrimp^{-1}$); FCR, food conversion ratio.
[3] Not significant ($P > 0.05$).

Discussion

Shrimp performance in relation to rearing system

In the present study, shrimp biological performance varied more strongly as a result of the rearing system than in relation to feed type. Nevertheless, the only deviation in shrimp performance between the experimental diets was found for shrimp body weight when animals were reared in green-water conditions.

At first, it appeared the green-water system imposed a greater challenge to shrimp growth when compared to the clear-water system. In outdoor tanks, considerable fluctuations in water salinity resulted in higher salinity levels compared to the indoor system (mean of 36.6 versus 32.4 g L^{-1}, respectively). However, clear-water tanks were intentionally deprived of natural food items and operated under a stocking density 1.7 times higher than the green-water tanks (100 versus 60 shrimp m^{-2}, respectively). At harvest, there was a slower growth (1.00 ± 0.06 g versus 1.04 ± 0.09 g $week^{-1}$, respectively) and lower survival (81.4 ± 7.3 versus 91.4 ± 5.4%, respectively) for shrimp reared in clear water than in green water. In clear-water tanks, shrimp yield was also higher which may have led to a greater build-up of nitrogen in the rearing system. These results and observations suggest that clear-water tanks promoted a more challenging culture environment for *L. vannamei*, despite the greater fluctuations in water quality observed in green-water conditions.

Differences in shrimp performance related to the experimental rearing system have been described in other studies (Leber & Pruder 1988; Tacon *et al.* 2002). Availability of naturally occurring food items, stocking density and water quality variation are the major parameters that lead to these differences. Tacon *et al.* (2002) compared the growth performance of *L. vannamei* over 8 weeks in an indoor running water culture system versus a zero-water exchange outdoor system. The authors found that shrimp reared in outdoor tanks grew 3.4 times faster than those stocked under indoor conditions. Tacon *et al.* (2002) explained the higher growth was primarily attributable to shrimp ability to obtain additional nutrients from food organisms endogenously produced within the zero-water exchange culture system.

In the present study, variation in shrimp final body weight observed in green water among experimental diets was likely to have been led by external factors such as water quality. As a matter of fact, water pH, temperature and salinity were 3.74 g L^{-1}, 0.40 and 2.18 °C, respectively, higher in green-water tanks than in the clear water ($P < 0.05$; *t*-test). As clear-water tanks were sheltered, there was little exposure to sunlight, maintaining water temperature below levels

observed in the green-water system. The higher exposure to sunlight also promoted a greater variation in water salinity under green-water conditions (coefficient of variation of 9.8% versus 7.8% for clear-water tanks). This fluctuation in salinity may have led to a higher osmoregulatory activity in shrimp reared in green water. Under these circumstances, some animals may have catabolized essential amino acids into energy to withstand a higher salinity concentration and fluctuation.

Overall, shrimp growth performance data are comparable to other studies conducted in similar rearing systems. Lemos & Nunes (2008) reported performance results for *L. vannamei* reared for 56 days at 114 shrimp m^{-2} in an indoor clear-water system. In their study, shrimp achieved a final survival ranging from 81.9 ± 9.26% to 93.8 ± 2.18% and weekly growth rates between 0.56 ± 0.10 g and 0.98 ± 0.14 g. Similarly in clear-water tanks in a time-restricted feeding study, Nunes *et al*. (2006) reported final shrimp survival from 92.3 ± 0.1% to 98.0 ± 0.01% and weekly growth from 0.51 ± 0.06 to 0.71 ± 0.01 g after 96 days of rearing. Tacon *et al*. (2002) working in 52-L glass aquariums stocked with *L. vannamei* at 100 shrimp m^{-2} for 8 weeks reported a final survival between 72.2% and 93.1% and weekly growth from 0.26 to 0.64 g.

Shrimp performance results obtained from the green-water culture system differed from other studies (Tacon *et al*. 2002; Izquierdo *et al*. 2006; Vasagam *et al*. 2009). These differences are attributed to bacterial floc production, shrimp stocking densities, feed composition and final shrimp survival rates. While most recent studies conducted with shrimp under outdoor tank conditions report the production of bacterial flocs with little or no water exchange, in the present study no attempt was made to promote bacterial growth. Instead, water was exchanged at a weekly basis and phytoplankton appeared to predominate in water. In a study conducted with the blue shrimp *Litopenaeus stylirostris* fed commercial diets from 440 to 520 g kg^{-1} CP, Vasagam *et al*. (2009) reported weekly growth rates between 1.19 and 2.46 g in a microcosm system stocked with 28 shrimp m^{-2}. Similarly, Tacon *et al*. (2002) working in microcosm tanks with microbial floc production reported that growth (and survival) of *L. vannamei* reared for 8 weeks at 51 shrimp m^{-2} varied from 1.11 g $week^{-1}$ (56.7%) to a maximum of 2.16 g $week^{-1}$ (69.7%). Izquierdo *et al*. (2006) working with the same stocking density, species and rearing system as Tacon *et al*. (2002) achieved a final shrimp survival above 96% after 56 days, but growth rates ranged from 0.83 ± 0.08 to 0.90 ± 0.03 g $week^{-1}$.

In general, the simultaneous use of two rearing systems with distinct characteristics in regard to water quality, natural food availability and shrimp stocking density acted as a valuable tool to better assess shrimp performance in regard to feed and nutrient composition. While the clear-water system acted as a better control to evaluate shrimp performance without the influence of environmental factors, it imposed a greater growth challenge because of a higher stocked shrimp biomass and the lack of naturally occurring food organisms. On the other hand, the green-water system was subjected to a greater variation in water quality, but increased water temperatures, natural food availability and lower stocked shrimp biomass enhanced shrimp growth and survival. In the present study, the green-water tanks better simulated farm rearing conditions, while the clear-water system strained shrimp growth performance under a more controlled environment.

Shrimp performance in relation to diet type

In the present study, there were few differences in shrimp biological performance as a result of diet type. The major variation was observed in final body weight when shrimp fed diets containing from 10 to 110 g kg^{-1} of krill meal (diets KM10, KM50 and KM110) were compared with those fed the basal and KO25 diets under the green-water system. As these differences were not statistically significant ($P > 0.05$) or replicated under a more controlled and challenging rearing condition, as found in clear-water tanks, it can be assumed they were partially the result of external factors (e.g. water quality) other than krill meal use.

Shrimp growth performance in this study indicated that krill meal and krill oil, plus increased dietary levels of meat and bone meal and soybean meal, were able to fully replace fish meal, soy lecithin or cholesterol nutritional value. Even at higher substitution values (i.e. diet KM110 without fish meal, soy lecithin or cholesterol and krill meal at 110 g kg^{-1} inclusion), shrimp displayed a normal growth, comparable to the basal diet, both in clear- and green-water rearing conditions.

However, contrary to other investigations with krill-derived products and penaeid shrimp, no added benefit in growth over the basal diet was achieved when krill meal or krill oil was used. Córdova-Murueta & García-Carreño (2002) fed *L. vannamei* juveniles with a re-pelleted 35%-CP commercial feed supplemented with krill hydrolysate (KH) at 3%, 9%, and 15% on a total CP basis. Authors reported that shrimp growth was higher at all supplementation levels than a control feed without KH. Smith *et al*. (2005) also observed a 20% higher growth rate and an improved FCR for *Penaeus monodon* when animals were fed on a 5% supplemented krill

meal diet. Williams *et al*. (2005) also working with *P. monodon* juveniles observed a superior growth for the krill meal-fed groups. Authors concluded that krill meal presents unknown growth factors capable of boosting shrimp growth. Williams *et al*. (2005) speculated that growth factors were residues of one or more bioactive neurosecretory hormones of the krill's X-organ–sinus gland complex.

The experimental design used in the present study significantly differed from the ones carried out by Córdova-Murueta & García-Carreño (2002), Smith *et al*. (2005) and Williams *et al*. (2005). The present study aimed at investigating the growth-related effect of replacing conventional protein and lipid sources used in penaeid diets by krill meal or krill oil. Contrary to the present study, these other studies investigated if krill meal and/or other derived products had the ability to stimulate shrimp growth and/or food consumption when used as a feed supplement. Another study would be required to look if the incorporation of krill meal and/or krill oil into the basal diet without any major ingredient replacement could provide a similar growth-promoting or feed intake effect as observed by Córdova-Murueta & García-Carreño (2002), Smith *et al*. (2005) and Williams *et al*. (2005).

In the clear-water conditions of the present study, there was slight increase in FCR when more krill meal was added to the diets (Table 3). This result can be partially explained by the positive effect that krill meal had on AFI. Krill meal acts as a strong feeding effector (Smith *et al*. 2005) assigned mainly to its high levels of proline, glucosamine and glycine, among others chemical compounds (Kolkovski *et al*. 2000) or to an insoluble protein present in its protein–chitin matrix (Williams *et al*. 2005). On the other hand, the higher feed intake was not efficiently converted into shrimp biomass. This may have been attributable to the expected higher content of chitin in diets containing more krill meal (KM50 and KM110).

Krill meal is a rich source of fluoride. Fluoride in krill meal comes mainly from its carapace. Previous studies with rats, freshwater organisms and fish have reported that excessive fluoride levels in dietary krill meal deteriorated animal growth and health (Zaleska-Freljan & Cywińska 1991; Metcalfe-Smith *et al*. 2003; Nankervis & Southgate 2006; Yoshitomi *et al*. 2006). These studies suggested that a maximum dietary fluoride level from krill meal exists beyond which clinical signs of toxicity can be detected. As opposed to these observations, in Atlantic salmon, *Salmo salar*, reared in seawater Julshamn *et al*. (2004) concluded that krill meal could be included up to 30% without any adverse effect on fish growth performance and survival. A possible explanation for the difference between krill's fluoride effect on marine and freshwater animals is their different adjustment to osmotic pressure (Moren *et al*. 2007; Yoshitomi *et al*. 2007). While freshwater animals need to keep minerals in their bodies to get osmotic homeostasis, marine animals, such as *L. vannamei*, work to release minerals as they live in a hyperosmotic environment.

In the present work, *L. vannamei* was reared in a water salinity of 32.4 ± 2.5 and 36.2 ± 3.5 g L^{-1}, respectively, under the clear-water and green-water rearing systems. Thus, although fluoride was ingested when shrimp were fed diets containing krill meal, animals actively worked to release it into the water. In the present work, in spite of the high concentration of fluorine in KM110, calculated as 165 mg kg^{-1} (Table 1), no detrimental effect was detected in *L. vannamei* growth performance. The maximum level of fluoride in KM110 (165 mg kg^{-1}) was far from exceeding the limit of 350 mg kg^{-1} imposed by the European Union (2008).

Results from the present work with *L. vannamei* is in agreement with the previous studies on fish, which also concluded that krill meal can partially replace fish meal without unfavourable effects on growth performance and health (Julshamn *et al*. 2004; Atlantic salmon; Suontama *et al*. 2007, Atlantic halibut). Although krill meal and krill oil are still more costly than fish meal and fish oil, respectively, their inclusion allowed withdrawing of expensive ingredients, namely soybean lecithin and cholesterol. Consequently, it is possible to achieve formula savings when krill meal and/or krill oil, plus increased levels of meat and bone meal and soybean meal, are used without detrimental effects to shrimp growth performance. Further work should focus on the benefits of key nutrients such as astaxanthin, phospholipids and highly unsaturated fatty acids present in krill meal and krill oil to shrimp growth performance and tail quality.

Acknowledgements

The first author acknowledges support from a research productivity fellowship (CNPq/MCT, PQ No. 300453/2009-4).

References

Akiyama, D.M., Dominy, W.G. & Lawrence, A.L. (1991) Penaeid shrimp nutrition. In: *Marine Shrimp Culture: Principles and Practices* (Fast, A.W. & Lester, L.J., eds), pp. 535–568. Elsevier, Amsterdam, Netherlands.

AOAC (2002) *Official Methods of Analysis of AOAC International*, 17th edn. Association of Official Analytical Chemists, Gaithersburg, USA.

CCAMLR, Convention on the Conservation of Antarctic Marine Living Resources (2000) *Report of the Nineteenth Meeting of the Scientific Committee*, pp. 1–261. Hobart, Australia.

Córdova-Murueta, J.H. & García-Carreño, F.L. (2002) Nutritive value of squid and hydrolysed protein supplement in shrimp feed. *Aquaculture*, **210,** 371–384.

European Union (2008) Directive 2008/76/EC of 25 July 2008. *Official Journal of the European Union*, **198,** 37–40.

FAO, Food and Agriculture Organization of the United Nations (2009) *The State of World Fisheries and Aquaculture 2008*. FAO Fisheries and Aquaculture Departament, Rome, Italy, 196 p.

Francis, G., Makkar, H.P.S. & Becker, K. (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, **199,** 197–227.

Gatlin, D.M. III, Barrows, F.T., Brown, P. *et al.* (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquac. Res.*, **38,** 551–579.

Glencross, B.D., Smith, D.M., Thomas, M.R. & Williams, K.C. (2002) Optimising the essential fatty acids in the diet for weight gain of the prawn, *Penaeus monodon*. *Aquaculture*, **204,** 85–99.

Hertramph, J.W. & Piedad-Pascual, F. (2000) *Handbook on Ingredients for Aquaculture Feeds*, 573 p. Kluwer Academic Publishers, Dordrecht, Netherlands.

Izquierdo, M., Forster, I., Divakaran, S., Conquest, L., Decamp, O. & Tacon, A. (2006) Effect of green and clear water and lipid source on survival, growth and biochemical composition of Pacific white shrimp *Litopenaeus vannamei*. *Aquac. Nutr.*, **12,** 192–202.

Julshamn, K., Malde, M.K., Bjorvatn, K. & Krogedal, P. (2004) Fluoride retention of Atlantic salmon (*Salmo salar*) fed Krill meal. *Aquac. Nutr.*, **10,** 9–13.

Kolkovski, S., Czeny, S. & Dabrowski, K. (2000) Use of Krill hydrolysate as feed attractant for fish larvae and juvenile. *J. World Aquac. Soc.*, **31,** 81–88.

Leber, K.M. & Pruder, G.D. (1988) Using experimental microcosms in shrimp research: the growth-enhancing effect of shrimp pond water. *J. World Aquac. Soc.*, **19,** 197–203.

Lemos, D. & Nunes, A.J.P. (2008) Prediction of culture performance of juvenile *Litopenaeus vannamei* by in vitro (pH-stat) degree of feed protein hydrolysis with species-specific enzymes. *Aquac. Nutr.*, **14,** 181–191.

Metcalfe-Smith, J.L., Holtze, K.E., Sirota, G.R., Reid, J.J. & de Solla, S.R. (2003) Toxicity of aqueous and sediment-associated fluoride to freshwater organisms. *Environ. Toxicol. Chem.*, **22,** 161–166.

Moren, M., Malde, M.K., Olsen, R.E., Hemre, G.I., Dahl, L., Karlsen, O. & Julshamn, K. (2007) Fluorine accumulation in Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), rainbow trout (*Oncorhynchus mykiss*) and Atlantic halibut (*Hippoglossus hippoglossus*) fed diets with Krill or amphipod meals and fish meal based diets with sodium fluoride (NaF) inclusion. *Aquaculture*, **269,** 525–531.

Nankervis, L. & Southgate, P. (2006) An integrated assessment of gross marine protein sources used in formulated microbound diets for barramundi (*Lates calcarifer*) larvae. *Aquaculture*, **257,** 453–464.

Nicol, S. & Foster, J. (2003) Recent trends in the fishery for Antarctic Krill. *Aquat. Living Resour.*, **16,** 42–45.

Nunes, A.J.P., Sá, M.V.C., Andriola-Neto, F.F. & Lemos, D. (2006) Behavioral response to selected feed attractants and stimulants in Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, **260,** 244–254.

Olsen, R.E., Suontama, J., Langmyhr, E., Mundheim, H., Ringø, E., Melle, W., Malde, M.K. & Hemre, G.-I. (2006) The replacement of fish meal with Antarctic Krill, *Euphausia superba*, in diets for Atlantic salmon, *Salmo salar*. *Aquac. Nutr.*, **12,** 280–290.

Smith, D.M., Tabrett, S.J., Barclay, M.C. & Irvin, S.J. (2005) The efficacy of ingredients included in shrimp feeds to stimulate intake. *Aquac. Nutr.*, **11,** 263–272.

Suontama, J., Karlsen, O., Moren, M., Hemre, G.-I., Melle, W., Langmyhr, E., Mundheim, H., Ringo, E. & Olsen, R.E. (2007) Growth, feed conversion and chemical composition of Atlantic salmon (*Salmo salar* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.) fed diets supplemented with Krill or amphipods. *Aquac. Nutr.*, **13,** 241–255.

Tacon, A.G.J., Cody, J.J., Conquest, L., Divakaran, S., Forster, I.P. & Decamp, O. (2002) Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. *Aquac. Nutr.*, **8,** 121–137.

Trathan, P.N., Watkins, J.L., Murray, A.W.A. *et al.* (2001) The CCAMLR-2000 Krill Synoptic Survey: a description of the rationale and design. *CCAMLR Sci.*, **8,** 1–24.

Vasagam, K.P.K., Suresh, A.V. & Chamberlain, G.W. (2009) Growth performance of blue shrimp, *Litopenaeus stylirostris* in self-cleaning microcosm tanks. *Aquaculture*, **290,** 236–242.

Watkins, J.L., Hewitt, R., Naganobu, M. & Sushin, V. (2004) The CCAMLR 2000 Survey: a multinational, multi-ship biological oceanography survey of the Atlantic sector of the Southern Ocean. *Deep-Sea Res. Pt. II*, **51,** 1205–1213.

Williams, K.C., Smith, D.M., Barclay, M.C., Tabrett, S.J. & Riding, G. (2005) Evidence of a growth factor in some crustacean-based feed ingredients in diets for the giant tiger shrimp *Penaeus monodon*. *Aquaculture*, **250,** 377–390.

Yoshitomi, B., Aoki, M., Oshima, S. & Hata, K. (2006) Evaluation of Krill (*Euphausia superba*) meal as a partial replacement for fish meal in rainbow trout (*Oncorhynchus mykiss*) diets. *Aquaculture*, **261,** 440–446.

Yoshitomi, B., Aoki, M. & Oshima, S.-I. (2007) Effect of total replacement of dietary fish meal by low fluoride krill (*Euphausia superba*) meal on growth performance of rainbow trout (*Oncorhynchus mykiss*) in fresh water. *Aquaculture*, **266,** 219–225.

Zaleska-Freljan, K. & Cywińska, L. (1991) The effect of difference Krill meals fed to laboratory rats on their blood indices. *Comp. Biochem. Physiol.*, **98A,** 133–136.