Effect of green and clear water and lipid source on survival, growth and biochemical composition of Pacific white shrimp *Litopenaeus vannamei*

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Abstract

Despite the shrimp ability to obtain additional nutrients from food organisms endogenously produced within the 'green water' system has been suggested as one of the causes for the better performance of Pacific white shrimp reared in 'green water' in comparison with 'clear water', the nutritional components responsible for these effects have yet to be determined. The present study aims to understand the importance of natural food organisms in zero-water exchange systems as source of essential fatty acids for the Pacific white shrimp *Litopenaeus vannamei*. Five treatments were tested: two conducted in mesocosms systems with shrimp-fed diets containing either fish oil (FO) or olive oil, and another three conducted in clear water with shrimp-fed diets containing either olive oil, a docosahexaenoic acid (DHA)-rich oil or an arachidonic acid (ARA)-rich oil. The presence of higher levels of fatty acids 16:1n-7, 17:1, 20:4n-6, 20:3n-3 and 22:5n-6, characteristic of floc lipids, in shrimp reared in mesocosms denoted their assimilation from the floc. Substitution of FO by olive oil in diets for shrimp reared in mesocosms did not affect growth or survival. Survival and growth of shrimp reared in mesocosms was better than those reared in clear water and fed an olive oil diet, whereas DHA or ARA enrichment of non-fish oil (NFO) diet improved survival of shrimp reared in clear water. Higher survival rate, triglyceride and DHA content in whole body and eyes of shrimp fed a DHA-rich diet suggests that under these conditions, in clear water, it is necessary to include at least 4.8 g kg^{-1} DHA in diet dry weight. ARA enrichment seemed to negatively affect growth. The nutritional contribution of the floc to shrimp in mesocosm culture reduces or eliminates the need for a dietary source of FO and illustrates the importance of DHA and ARA to enhance shrimp survival in clear water conditions.

KEY WORDS: arachidonic acid, docosahexaenoic acid, fatty acids, green water, mesocosms, Pacific white shrimp

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Abbreviations: EFA, essential fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ARA, arachidonic acid; HUFA, highly unsaturated fatty acids with 20 or more carbon atoms and three or more double bonds.

Introduction

Although most of the global shrimp production is based in extensive pond culture systems, there is increasing pressure on the shrimp-farming community to improve biosecurity and effluent control. Completely closed farming systems, with no water exchange except that added to make up for evaporative losses, also reduce costs and water use, improving sustainability and environmental compatibility. Production of Pacific white shrimp (*Litopenaeus vannamei*) in such systems has demonstrated the advantage of faster growth rates than those obtained in intensive 'clear water' systems (Tacon *et al.* 2002). Several factors may be involved in the beneficial effect of 'green water' for shrimp growth such as water quality control, microbial community composition, nutrient source, shading, enzyme enhancement, etc. Organisms present in mesocosms contribute to biological waste treatment by utilizing and removing potentially toxic faecal wastes and metabolites from the water. Thus, even algae with low nutritional value are useful in keeping the N and P content low and improving the survival rate of shrimp larvae (Okauchi *et al.* 1997). Other studies have demonstrated that maintenance of the natural microbial community is highly

beneficial for the survival of shrimp (Alabi *et al*. 1999). Natural food organisms found in these mesocosms systems; mainly bacteria, phytoplankton, protozoans and metazoans could also play a key role in the nutritional budget and health of pond-farmed shrimp. Thus, the ability of shrimp to obtain additional nutrients from food organisms endogenously produced within the 'green water' system has been suggested as one of the causes for the better growth and feed performance of Pacific white shrimp reared in outdoor 'green water' in comparison with indoor 'clear water' (Tacon *et al*. 2002). The nutritional components responsible for these effects have yet to be determined, as there is insufficient information of the nutritional requirements of this species and the compounds and mechanisms produced by the floc to enhance culture performance.

Regarding the dietary lipid component, several studies have demonstrated the essentiality of linoleic (LA; 18:2n-6) and linolenic (LNA; 18:3n-3) acids for normal growth and survival of penaeid shrimp (*Fenneropenaeus indicus*, Colvin 1976; *Marsupenaeus japonicus*, Kanazawa *et al*. 1979; *Penaeus monodon*, Merican & Shim 1997; Glencross & Smith 1999; *F. chinensis*, Xu *et al*. 1994), as well as of docosahexaenoic (DHA) and eicosapentaenoic acids (EPA; Colvin 1976; Kanazawa *et al*. 1979; Glencross & Smith 2001a) due to the low ability of these crustaceans to synthesize long chain fatty acids from their 18 carbon atoms precursors (Teshima *et al*. 1992). More recently, the importance of DHA for different penaeid species has been emphasized by several authors (Teshima 1998; González-Félix *et al*. 2003), with levels of this fatty acid in muscle (Araujo & Lawrence 1993) and ovaries (Wouters *et al*. 2001) of wild *L. vannamei* accounting for 7.8% and 7.1–9.8% of the total fatty acids respectively. However, few studies have been conducted on the effect of dietary DHA on Pacific white shrimp culture.

Although arachidonic acid (ARA) is also considered an essential fatty acid (EFA) for marine fish (Castell *et al*. 1994; Bessonart *et al*. 1999; Izquierdo *et al*. 2000), controversy exists about its essentiality for different shrimp species (Glencross & Smith 2001b). ARA supplementation of *F. chinensis* (Xu *et al*. 1994) diets significantly enhanced growth, whereas there was a negative dose–response effect on growth of *P. monodon* (Glencross & Smith 2001b). In *L. vannamei* very high levels of ARA are found in muscle of wild shrimp, reaching those of DHA, but about half of those EPA (Araujo & Lawrence 1993). Similarly, ARA levels in the ovaries of wild females of this species showed a high ARA content, similar to that of DHA but half that of EPA (Wouters *et al*. 2001). Lately, in clear water reared juvenile *L. vannamei*, González-Félix *et al*. (2003) found improved growth with 5 g kg^{-1} ARA added to formulated diets.

The present study is included in a series of studies conducted by the Nutrition Department of the Oceanic Institute in Waimanalo, Hawaii, to understand the importance of floc in zero-water exchange systems as a source of EFAs for the Pacific white shrimp. Thus, the main objective of this study was to evaluate the potential of green water nutrients as source of DHA and ARA, determining the evolution of floc fatty acid profile along the feeding experiment, the possible incorporation of EFAs from floc into shrimp tissues and the effect of dietary DHA and ARA fed in a clear water system.

Materials and methods

Shrimp were fed initially a 520 g kg^{-1} protein commercial larval shrimp diet (Higashimaru Co. Ltd, Kagoshima, Japan) and later a 350–400 g kg^{-1} protein commercial nursery shrimp diet (Rangen, Inc., Buhl, ID, USA). The shrimp (0.71 ± 0.02 g individual weight) were randomly stocked in 15 outdoor black tanks (1300 L working volume) at 100 shrimp per tank (77 shrimp m^{-3}) and fed either a diet (Table 1) containing fish oil (FO mesocosms), and hence a high amount of DHA and EPA EFAs, or olive oil (non-fish oil, NFO mesocosms), which contains low levels of potential EFAs for this species such as LA, LNA, ARA, EPA or DHA. To develop a microbial community, these treatments (FO mesocosms and NFO mesocosms), were innoculated with 10 L of water from an established shrimp culture system with a diatom predominant floc community, 3 days prior to the shrimp stoking. Zero-saltwater exchange was maintained for the duration of the trial, using freshwater to replace evaporative loss and maintain salinity levels (32–35 g L^{-1}). In another three treatments, clear water was used in a flow through system, tanks were covered to avoid phytoplankton growth and shrimp were fed diets containing either olive oil (NFO clear water), a DHA-rich oil (DHA clear water) or an ARA-rich oil (ARA clear water). All diets contained 300 g kg^{-1} crude protein and 80 g kg^{-1} lipids (Table 1), and were offered to shrimp on a 24 h basis using a feeding belt for 56 days. Feed ration size was determined based on shrimp size and water temperature, recommended by Tacon *et al*. (2002). Each treatment was simultaneously tested by three replicates. Dissolved oxygen, temperature and turbidity of the culture water were measured daily; pH, salinity, nitrate-N, nitrite-N, total ammonia nitrogen (TAN), chlorophyll *a* and total suspended particulate matter were measured weekly. Periodic monitoring of dominant taxa of phytoplankton and protozoans was performed

Table 1 Composition of experimental diets

Ingredient (g kg^{-1})	Diet			
	FO	NFO	DHA	ARA
Fishmeal – LT 94[1]	110	110	110	110
Whole hard red winter wheat[2]	550.0	550.0	570.5	576.5
Squid Liver Powder[3]	25.0	25.0	25.0	25.0
Wheat gluten[2]	40.0	40.0	40.0	40.0
Brewers yeast[4]	50.0	50.0	50.0	50.0
Soybean meal, dehulled solvent extracted[5]	150.0	150.0	110.0	110.0
Advantage®[6]	0.0	0.0	25.0	0.0
Arachidonic acid®[6]	0.0	0.0	0.0	20.0
Soy lecithin – Aqualipid 95	20.0	20.0	20.0	20.0
Menhaden oil[7]	35.9	0.0	0.0	0.0
Cholesterol-SF[8]	2.3	2.3	2.3	2.3
Olive oil	0.0	35.8	30.4	29.3
Potassium phosphate, dibasic[9]	5.6	5.6	5.6	5.6
Sodium phosphate, dibasic[9]	5.6	5.6	5.6	5.6
Magnesium phosphate, dibasic[9]	5.6	5.6	5.6	5.6

[1] SSF Sildolje-og Sildememlindustiens Forskningsinstitute, Norway.
[2] Hawaii Flour Mill, Honolulu, HI, USA.
[3] Milae, South Korea.
[4] Williams Bio-Products, Decatur, IL, USA (by courtesy).
[5] Land-O-Lakes, Seattle, WA, USA.
[6] AquaGrow, Aqua-In-Tech, Inc., Lynnwood, WA, USA (by courtesy).
[7] Omega Protein, Reedville, VA, USA.
[8] Solvay Pharmaceuticals B.V., Veenendaal, the Netherlands.
[9] ICN Biomedicals, Inc., Aurora, OH, USA.

during the last 6 weeks of the trial. Water temperature along the experimental period ranged from 24.2 to 29 °C, without main or constant differences among dietary treatments. All shrimp were weighed at the start and termination of the trial with biweekly intermediate weightings of 20 shrimp subsamples. Shrimp biomass, survival, final body weight, weight gain, specific growth rates (SGR) and feed conversion ratios (FCR) were calculated from these data. To obtain floc samples, 5 L of tank water were weekly collected from each replicate, immediately centrifuged to remove the clear water and stored at −80 °C for proximate and fatty acid analysis. At the end of the trial, the remaining floc in the tank water was also collected, processed and stored for analysis. Two samples of 30 shrimp each, for whole body and eye composition, common to all treatments at the initiation of the trial and another two samples of 10 shrimp each, for whole body and eye composition, from each tank at the end of the trial were collected and stored at −80 °C until dissection and analysis. Dissected eyes and whole body samples were pooled for each tank. Samples of the diets were also analysed.

Moisture and protein content of diets, floc and animals were determined (AOAC 1995) and crude lipids were extracted as described by Folch *et al.* (1957) in an acid medium (1 M HCl was added to the chloroform : methanol mixture to obtain a final concentration of 68 mM HCl), to facilitate lipid extraction, particularly from floc organisms bearing cell wall. Dietary lipids were separated by thin layer chromatography in S-II Chromarods, using methyl acetate : isopropanol : chloroform : methanol : 0.25% KCl (25 : 25 : 25 : 10 : 9) as solvent, and quantified by Iatroscan (Iatron Lab Inc., Tokyo, Japan), triplicate analysis being conducted for each sample. Fatty acid methyl esters were obtained by *trans*-esterification with 1% sulphuric acid in methanol (Christie 1989) using heneicosaenoic acid (10% of total lipids) as internal standard. The fatty acid methyl esters obtained were separated by gas chromatography (Shimadzu GC-14 a, Kyoto, Japan) run at the operation conditions described by Izquierdo *et al.* (1990), quantified by an FID detector, and identified by comparison with well characterized external standards.

The data analysis were carried out by a General Linear Model (GLM), using STATGRAPHICS software (STATGRAPHICS Plus for Windows 3.1; Manugistics, Inc., Rockville, MD, USA). The variables measured in percentage were normalized by the arcsine transformation and all data were tested for normality and homogeneity of variances.

The simple GLM used was as follows:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij},$$

where μ is the population mean, α_i the fixed effect of the diet i and ε_{ij} the residual error.

Weight data were analysed using a nested GLM, such that:

$$Y_{ijk} = \mu + \alpha_i + \beta_{ij} + \varepsilon_{ijk},$$

where μ is the population mean, α_i the fixed effect of the diet i, β_{ij} random effect of the tank j within the diet i and ε_{ijk} the residual error.

Results

Chlorophyll a, total suspended solids and physico-chemical parameters

Levels of chlorophyll *a* in the culture water gives an indication of phytoplankton biomass; total suspended solids provides a quantification of the overall floc in the tank including aggregates of bacteria, phytoplankton, protozoans, metazoans and detritus. Water-column chlorophyll *a* levels between the FO and NFO treatments were not statistically different ($P = 0.05$). Over the last 5 weeks of trial, culture systems had stable phytoplankton bloom averaging 44 μg L^{-1} chlorophyll *a* and varying by about 9%. Total suspended solids levels between the treatments were neither statistically different. The amount of floc particulates over the trial period ranged from 50 to 200 mg L^{-1}. Over the course of the trial the mean water temperature was 26.4 °C (range: 22.5–29.7), mean pH was 7.9 (range: 7.5–8.2) and mean dissolved oxygen was 6.8 (5.8–7.7) mg L^{-1}. Dissolved inorganic nutrient levels (TAN, nitrite and nitrate) between the treatments were not different, although the covered/flow through treatments showed less variability in levels throughout the trial than did the uncovered/zero-exchange treatments. The highest levels attained in all treatments were 0.2, 0.14 and 2.2 mg L^{-1} for TAN, nitrite and nitrate respectively. These are levels are well within normal parameters for culture of this species. There was no evident effect of treatment on any of these parameters.

Fatty acids in diets, floc and shrimp

All experimental diets contained similar levels of 18:2n-6 [average 2.3% on a dry weight basis (d.b.)] and 18:3n-3 (average 0.23% d.b.; Table 2). As expected, diet FO had the highest polyunsaturated fatty acid contents, particularly regarding EPA (0.522% d.b.) and DHA (0.61% d.b.; Table 2), whereas the other three diets were rich in monounsaturated fatty acids (>3% and only 2.17 in FO diet). Diet NFO contained the lowest EPA (0.132% d.b.) and DHA (0.262% d.b.) levels. The DHA diet was high in DHA (0.479% d.b.) and ARA diet showed the highest ARA (0.081% d.b.) contents. EPA/ARA values were high in FO, NFO and DHA diets (12.27–14.45) and low in ARA diet (1.6). Floc biochemical composition varied along the experiment and among replicates. In average, the fatty acid composition of floc total lipids was similar except for the higher n-9 fatty acids content in floc from FO tanks and higher 22:3n-6, 22:4n-6 and 22:5n-6 together with lower 18:2n-6 in floc from NFO tanks (Table 2). Floc lipids of both types were characterized by high amounts of 16:0, 16:1n-7, 18:1n-9, 18:3n-3, 20:5n-3 and 22:6n-3. Additionally, they were higher in 16:1n-7, 17:1, 20:4n-6, 20:3n-3 and 22:5n-6 than the FO and NFO diets. EPA/ARA values in floc were lower than in FO, NFO and DHA diets, but 1.4–1.9 times higher than those in ARA diet.

Survival of shrimp reared in mesocosms was over 95% and was not affected by diet (Table 3). Survival of shrimp reared in clear water and fed the NFO diet was significantly lower (56.7%), but shrimp fed the DHA- or ARA-rich diets in clear water had intermediate survival rates (>75%). Growth (body weight) cumulative weight and SGR of shrimp cultured in mesocosms, regardless of the diet fed (Table 3) more than doubled that reported in commercial farms (McIntosh & Carpenter 1999). Higher variability was found in body weight, weight gain and SGR in mesocosms-cultured shrimp-fed diet NFO than those fed diet FO. From the third week of feeding on, SGRs were significantly higher in shrimp cultured in mesocosms, regardless of the diet fed (Fig. 1), than that of shrimp reared in clear water and fed ARA diet. SGR and final body weight were significantly better for shrimp reared in mesocosms (Table 3, Fig. 2) than those reared in clear water, while FCR values of shrimp in mesocosms were only significantly better than those fed NFO diet in clear water. In clear water, dietary inclusion of DHA or ARA slightly improved FCR, in comparison with shrimp-fed NFO, but did not significantly improve growth, although the final biomass of shrimp produced was significantly higher when DHA was supplemented in the diet in comparison with shrimp-fed NFO diet.

Whole body lipid content was slightly lower when shrimp were cultured in clear water system, particularly when fed the NFO diet (Table 4). Shrimp fed the NFO diet in clear water had a significantly lower relative content of triglycerides and slightly higher relative content of phosphoacylglycerides in whole body lipids, whereas cholesterol, cholesterol esters and free fatty acid contents were not affected.

Table 2 Fatty acid content of shrimp diets and floc ($n = 3$; % dry weight)

Fatty acid	FO diet	NFO diet	DHA diet	ARA diet	FO floc	NFO floc
14:0	0.345	0.106	0.170	0.126	0.173	0.216
14:1	0.002	0.002	0.002	0.002	0.086	0.077
15:0	0.026	0.011	0.009	0.011	0.018	0.025
15:1	n.d.	n.d.	n.d.	n.d.	0.024	0.021
16:0iso	0.004	n.d.	n.d.	n.d.	0.029	0.014
16:0aniso	0.004	0.002	0.002	0.002	0.068	0.072
16:0	1.551	1.627	1.379	1.398	1.836	1.620
16:1n-9	n.d.	n.d.	n.d.	n.d.	0.191	0.308
16:1n-7	0.475	0.119	0.119	0.120	0.665	0.875
16:1n-5	0.016	0.005	0.005	0.003	0.023	0.022
16:2	0.067	0.008	0.008	0.010	0.055	0.082
17:0	0.036	0.013	0.011	0.014	0.016	0.011
17:1	0.081	0.010	0.010	0.011	0.131	0.182
16:4n-1	0.004	n.d.	n.d.	n.d.	0.099	0.009
16:4n-3	0.034	0.005	0.004	0.005	0.025	0.024
18:0	0.248	0.309	0.251	0.302	0.275	0.165
18:1n-11	n.d.	n.d.	n.d.	n.d.	n.d.	0.013
18:1n-9	1.253	3.058	2.927	2.784	1.043	0.354
18:1n-7	0.006	n.d.	n.d.	0.019	0.264	0.197
18:2n-9	0.003	n.d.	n.d.	0.004	n.d.	n.d.
18:2n-6	2.214	2.402	2.357	2.365	0.630	0.185
18:3n-9	0.020	0.003	n.d.	n.d.	n.d.	n.d.
18:3n-6	0.017	0.003	0.013	0.012	0.037	0.041
18:4n-6	0.027	n.d.	0.008	0.003	n.d.	n.d.
18:3n-3	0.257	0.233	0.220	0.219	0.212	0.219
18:4n-3	0.119	0.033	0.030	0.030	0.118	0.103
20:0	0.012	0.020	0.017	0.020	0.014	0.008
20:1n-9	0.180	0.141	0.154	0.162	0.067	0.012
20:1n-7	0.012	0.002	0.005	n.d.	0.005	0.007
20:2n-9	0.009	n.d.	0.002	0.003	n.d.	n.d.
20:2n-6	0.012	0.008	0.008	0.009	0.058	0.008
20:3n-6	0.009	0.003	n.d.	0.011	0.014	0.013
20:4n-6	0.036	0.010	0.011	0.081	0.101	0.132
20:3n-3	0.002	0.002	n.d.	0.002	0.010	0.010
20:4n-3	0.049	0.007	0.007	0.009	0.007	0.008
20:5n-3	0.522	0.127	0.132	0.130	0.305	0.296
22:0	0.025	0.017	0.014	0.024	0.013	0.010
22:1n-11	0.218	0.146	0.182	0.186	0.014	0.011
22:1n-9	n.d.	n.d.	n.d.	n.d.	0.025	0.011
22:3n-6	0.031	0.007	0.006	0.011	0.003	0.019
22:4n-6	0.016	0.005	0.004	0.012	0.007	0.022
22:5n-6	0.022	0.008	0.007	0.010	0.022	0.034
22:4n-3	0.014	0.006	0.008	0.007	n.d.	n.d.
22:5n-3	0.084	0.005	0.015	0.018	0.018	0.021
24:0	0.019	0.017	0.016	0.028	0.013	0.013
22:6n-3	0.610	0.262	0.479	0.337	0.231	0.101
Saturated	2.267	2.122	1.878	1.924	2.426	2.141
n-3	1.690	0.678	0.895	0.757	0.926	0.782
n-6	2.384	2.445	2.412	2.515	0.871	0.454
n-9	1.465	3.202	3.083	2.954	1.136	0.377
n-3 HUFA	1.280	0.408	0.640	0.503	0.571	0.436
EPA/ARA	14.45	12.31	12.27	1.60	3.02	2.25
EPA/DHA	0.86	0.48	0.28	0.39	1.32	2.94
Crude lipids	8.70	8.74	8.60	8.50	6.42	5.34

n.d., not detected.

The fatty acid composition of total lipids from whole body of shrimp showed the highest EPA, DHA and n-3 highly unsaturated fatty acids with 20 or more carbon atoms and three or more double bonds (HUFA) content for the shrimp fed the FO diet in mesocosms and the lowest in shrimp fed the NFO diet in clear water (Table 5). In comparison,

Table 3 Growth, survival and feed utilization of shrimp (0.71 ± 0.02 g initial weight) cultured in mesocosms or clear water and fed for 56 days different types of lipid sources (mean ± SE, $n = 3$)

	FO mesocosms	NFO mesocosms	NFO clear water	DHA clear water	ARA clear water
Survival (% initial shrimp)	96.0 ± 1.0 a	96.3 ± 3.1 a	56.7 ± 22.0 b	76.0 ± 15.5 ab	81.7 ± 3.2 ab
Final body weight (g)	7.88 ± 0.22 a	7.40 ± 0.61 a	3.75 ± 0.66 b	3.73 ± 0.24 b	2.96 ± 0.17 b
Mean weekly growth (g)	0.90 ± 0.03 a	0.83 ± 0.08 a	0.38 ± 0.08 b	0.38 ± 0.03 b	0.28 ± 0.02 b
Cumulative gain (%)	1039.4 ± 13.1 a	921.1 ± 81.0 a	432.7 ± 91.7 b	416.5 ± 31.8 b	316.6 ± 18.7 b
SGR	4.34 ± 0.02 a	4.15 ± 0.14 ab	2.97 ± 0.32 ab	2.93 ± 0.11 ab	2.55 ± 0.08 b
FCR (feed/biomass gain)	1.24 ± 0.07 a	1.27 ± 0.06 a	4.66 ± 2.60 b	2.80 ± 0.80 ab	3.00 ± 0.25 ab

Different letters in the same row denote significant differences ($P < 0.05$).

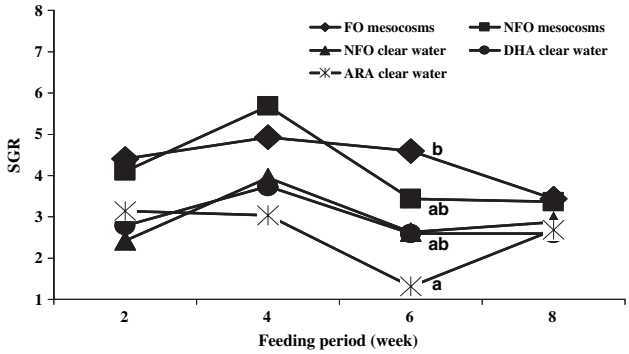

Figure 1 Specific growth rate at different periods of shrimp-fed diets containing different oils.

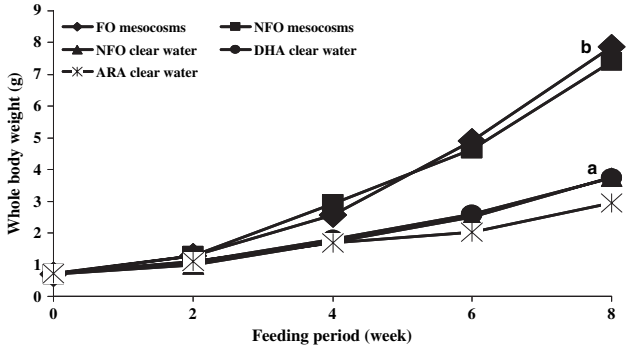

Figure 2 Whole body weight versus time of shrimp-fed diets containing different lipids.

feeding the NFO diet in the mesocosm system enhanced the incorporation of EPA, DHA, n-3 HUFA and ARA into the body lipids, whereas DHA and ARA diets enhanced DHA and ARA incorporation respectively. Mesocosm-reared shrimp were higher in saturated fatty acids as well as in 16:1n-7, 17:1, 20:3n-3 and 22:5n-6 than those reared in clear water. Mesocosm-reared shrimp were also higher in 20:4n-6 fatty acid than were clear water cultured shrimp, except for those fed the ARA diet. The lowest EPA/ARA ratios were found in shrimp fed the ARA diet.

Lipid levels in shrimp eyes were several times higher than what was found in the whole body. Shrimp cultured in clear water systems had low eye lipid levels, whereas shrimp reared in mesocosms had significantly increased eye lipid content, even when NFO diet was fed (Table 6). In comparison with whole body lipids, fatty acid composition of shrimp eyes was slightly higher in the relative proportion of saturated fatty acids (32.73% and 20.95% total fatty acids, for eyes and body respectively) and lower in monounsaturated ones (27.35% and 29.8% total fatty acids, respectively). Highest EPA, DHA and n-3 HUFA contents were found in eyes of shrimp reared in mesocosms (Table 6). Feeding the NFO diet in the mesocosm system enhanced the incorporation of EPA, DHA, n-3 HUFA and ARA into the eye lipids, despite their low levels in this diet, and feeding DHA and ARA diets enhanced DHA and ARA incorporation in shrimp eyes to a higher extent than in whole body lipids. Eye lipids of mesocosm-reared shrimp were higher in saturated fatty acids as well as in 16:1n-7, 17:1 and 22:5n-6 than shrimp cultured in clear water. ARA content in lipid eyes from mesocosm-reared shrimp were also higher than in clear water shrimp, except for those fed the ARA diet. The lowest EPA/ARA ratios in eyes lipids were found in shrimp fed the ARA diet.

Discussion

Dietary lipid composition seemed to have affected lipid content and fatty acid profile of the floc, denoting the interaction between both components of the mesocosms system. Community succession could also contribute to the differences found in floc along the experiments. The richness of the floc in 16:0, 16:1n-7 and 18:1n-7 fatty acids suggests lipid contribution of microbial communities from sludge, whereas the high ratio of 16:0/16:1n-7 suggests a poor contribution of diatoms or flagellates (Napolitano 1990). Contribution of lipids to floc composition was up to 2.5 times higher than that described by Tacon *et al.* (2002) for floc obtained in the same culture system and with similar diets.

Table 4 Lipid content (g kg^{-1}) and composition of lipid classes (% total lipids) from shrimp whole body lipids (mean ± SE)*

	Crude lipids	Cholesterol esters	Triglycerides	Free fatty acids	Free esterol	Polar lipids
Initial	18.70 ± 1.50	9.50 ± 0.65	3.77 ± 0.98	9.98 ± 0.70	16.37 ± 0.64	59.97 ± 2.23
FO mesocosms	29.10 ± 0.70	3.69 ± 0.77	16.88 ± 1.52 a	6.91 ± 0.77	14.69 ± 0.75	57.02 ± 3.13
NFO mesocosms	28.60 ± 1.30	3.90 ± 0.78	13.08 ± 0.71 a	6.20 ± 0.74	16.32 ± 1.05	58.42 ± 0.95
NFO clear water	21.20 ± 3.20	2.94 ± 0.68	5.86 ± 1.79 b	6.98 ± 1.25	12.97 ± 3.16	70.09 ± 7.59
DHA clear water	22.80 ± 3.80	3.30 ± 0.45	12.85 ± 0.62 a	5.80 ± 0.72	15.72 ± 0.45	61.20 ± 0.85
ARA clear water	20.40 ± 1.20	2.77 ± 0.35	12.50 ± 0.40 a	5.31 ± 0.78	15.00 ± 0.28	63.36 ± 0.89

*$n = 3$, different letters among lipid values of shrimp from different treatments indicate significant differences ($P < 0.05$).

This observation, together with the higher contents on 16:0, 16:1n-7 and 18:1n-7 fatty acids, denotes a more effective lipid extraction from cell wall-bearing organisms by the acid medium used in the present experiment, than the traditional Folch method used previously.

The presence of higher 16:1n-7, 17:1, 20:4n-6, 20:3n-3 and 22:5n-6 fatty acid levels, characteristic of floc lipids from both tank types, in shrimp reared in the mescosms system denoted the assimilation of these type of nutrients from the floc. Substitution of FO by olive oil in diets of shrimp reared in the mesocosm system did not affect growth or survival, and only reduced shrimp n-3 HUFA contents by 48%, in contrast with the 68% n-3 HUFA reduction in the diets, suggesting the incorporation of these EFA from the floc as it occurred with the other fatty acids. Indeed, if 16:1n-7 and 17:1 were effectively assimilated from the floc by shrimp, it is reasonable to assume that the rate of assimilation of n-3 HUFAs was also high, in view of their higher digestibility in penaeids (Merican & Shim 1994).

Mesocosm culture conditions significantly improved survival and growth, regardless of the diet fed, of shrimp reared in clear water and fed a NFO diet. The improved survival of shrimp-fed diets containing DHA or ARA in clear water in comparison with those fed olive oil as the sole lipid source, also supports the hypothesis that the presence of those EFA in the floc are, at least partly, responsible for the better performance of shrimp in mesocosms than in clear water. Nevertheless, other factors, including mineral and vitamin contribution of floc to shrimp nutrition, must have been the cause of the lower growth and survival of shrimp fed a diet without FO supplementation in clear water in comparison with mesocosms. Low survival and growth performance of shrimp fed a diet without FO supplementation in clear water induced a low content of triglycerides, possibly accumulated in the gastric gland in agreement with the reduced lipid deposition in this organ in shrimp fed reduced dietary lipid levels (D'Abramo *et al.* 1989). In general, *L. vannamei* lipid classes composition of shrimp from the other treatments was similar to that of *M. japonicus* (Teshima *et al.* 1987) and *P. monodon* (O'Leary & Matthews 1990), being slightly lower in free sterols than the latter one.

Shrimp eye lipid contents were seven times higher than in the whole body and were more affected by the culture system used. Lipids, including EFAs such as DHA, phosphoglycerides and vitamin A, have long been recognized to be very important in the development of fish eyes (Kanazawa 1993; Sargent *et al.* 1993; Izquierdo *et al.* 2000; F.J. Roo, M.S. Izquierdo, J. Socorro, C.M. Hernández-Cruz and A. Valencia, personal communication). DHA and EPA were particularly high in shrimp eyes, regardless of the diet used, and despite the relatively high DHA/EPA dietary ratio, suggesting the importance of both fatty acids for shrimp's eyes. However, the fatty acid composition of the eyes was less affected by the diet than was the whole body. Similarly, low levels of dietary EFAs in rotifers fed to gilthead seabream did not cause a drastic alteration in eye's EFA content but induced a reduction in eye diameter and cone photoreceptor density (Izquierdo *et al.* 2000; F.J. Roo, M.S. Izquierdo, J. Socorro, C.M. Hernández-Cruz and A. Valencia, personal communication).

Higher survival rate, TG and DHA content in whole body and eyes of shrimp fed a DHA-rich diet suggest that, in clear water, at least 4.8 g kg^{-1} DHA in diets (dry weight) containing about 1.3 g kg^{-1} EPA and 0.1 g kg^{-1} ARA (6.4 g kg^{-1} n-3 HUFA) should be included in the diet. This is in agreement with González-Félix *et al.* (2004) who recommended a minimum dietary n-3 HUFA content of 5 g kg^{-1} (dry weight) for Pacific white shrimp reared in clear water and intensive conditions. These requirements are lower than those determined for *M. japonicus* (10 g kg^{-1} DHA or 10 g kg^{-1} n-3 HUFA, Kanazawa *et al.* 1977, 1979), *F. chinensis* (5–10 g kg^{-1} DHA, Xu *et al.* 1994) and *P. monodon* (9 g kg^{-1}% DHA, Glencross & Smith 2001a). DHA has been found to be particularly important for penaeids during reproduction (Millamena *et al.* 1993; Cahu *et al.* 1994). However, the sole elevation of this fatty acid in diets for shrimp in the present experiment was insufficient to promote growth. Although other nutrient deficiencies in clear water

Table 5 Average fatty acid content of total lipids from whole body of shrimp cultured in either mesocosms or clear water system and fed several lipid sources ($n = 3$; % dry weight)

Fatty acid	Initial	FO mesocosms	NFO mesocosms	NFO clear water	DHA clear water	ARA clear water
14:0	0.021	0.031 a	0.013 ab	0.005 b	n.d.	0.010 ab
14:1	n.d.	0.004	n.d.	n.d.	n.d.	n.d.
15:0	n.d.	0.011 a	0.006 ab	0.003 b	n.d.	0.003 b
15:1	0.006	0.007	n.d.	n.d.	n.d.	n.d.
16:0iso	n.d.	0.017	0.018	0.016	0.019	0.015
16:0aniso	0.022	0.028	n.d.	n.d.	n.d.	0.021
16:0	0.431	0.675 a	0.544 a	0.351 b	0.407 b	0.392 b
16:1n-9	n.d.	n.d.	n.d.	n.d.	n.d.	0.003
16:1n-7	0.035	0.077 a	0.030 b	0.011 c	0.012 c	0.017 c
16:2	0.019	0.019	0.004	0.008	0.007	0.011
17:0	n.d.	0.019	0.013	n.d.	n.d.	n.d.
17:1	0.004	0.007	0.005	n.d.	n.d.	n.d.
16:4n-1	n.d.	0.036	0.030	0.031	0.036	0.037
16:4n-3	0.003	0.004	n.d.	n.d.	n.d.	n.d.
18:0	0.187	0.234 a	0.171 ab	0.141 b	0.145 b	0.169 b
18:1n-9	0.174	0.291 d	0.855 a	0.668 b	0.692 b	0.473 c
18:1n-7	0.063	0.090	n.d.	n.d.	n.d.	0.047
18:2n-6	0.139	0.375	0.493	0.445	0.435	0.351
18:3n-6	0.004	0.007	0.006	0.004	n.d.	0.004
18:4n-6	n.d.	0.005	n.d.	n.d.	n.d.	n.d.
18:3n-3	0.013	0.026	0.029	0.022	0.019	0.018
18:4n-3	n.d.	0.008	0.003	n.d.	n.d.	n.d.
20:0	0.007	0.008	0.009	0.005	0.006	0.007
20:1n-9	0.023	0.042	0.056	0.040	0.051	0.032
20:1n-7	0.003	0.007	0.004	0.003	n.d.	n.d.
20:2n-6	0.012	0.032	0.034	0.036	0.046	0.030
20:3n-6	0.003	0.004	n.d.	n.d.	n.d.	0.002
20:4n-6	0.063	0.061 a	0.054 a	0.020 b	0.015 b	0.069 a
20:3n-3	0.003	0.006	0.005	n.d.	n.d.	0.003
20:4n-3	0.004	0.009	0.003	n.d.	n.d.	n.d.
20:5n-3	0.304	0.341 a	0.177 b	0.113 c	0.129 bc	0.167 b
22:0	0.010	0.016	0.013	0.006	0.008	0.010
22:1n-11	n.d.	0.023	0.040	0.019	0.028	0.011
22:3n-6	0.003	0.021	0.023	n.d.	n.d.	n.d.
22:4n-6	n.d.	0.045	n.d.	n.d.	n.d.	n.d.
22:5n-6	0.004	0.038 a	0.034 a	0.004 b	n.d.	n.d.
22:5n-3	0.016	0.040 a	0.020 b	0.006 c	0.005 c	0.006 c
22:6n-3	0.285	0.361 a	0.195 b	0.166 c	0.220 b	0.192 b
Saturated	0.686	1.004 a	0.782 a	0.528 b	0.585 b	0.607 b
Monounsaturated	0.302	0.508 c	0.986 a	0.739 b	0.783 ab	0.547 c
n-3	0.629	0.786 a	0.425 ab	0.307 b	0.372 b	0.385 b
n-6	0.228	0.552	0.636	0.506	0.497	0.452
n-3 HUFA	0.613	0.756 a	0.395 b	0.286 c	0.353 b	0.367 b
EPA/ARA	4.85	5.55	3.27	5.70	8.43	2.43
EPA/DHA	1.07	0.96	0.92	0.68	0.59	0.86

Different letters in the same row denote significant differences ($P < 0.05$).
n.d., not detected.

systems may also have interfered with growth, the lack of effect of DHA on growth may be related to the low EPA levels in the diet (1.3 g kg^{-1}, EPA/DHA: 0.28) in view of the high contents that Pacific shrimp shows in wild populations (EPA/DHA: 2; Araujo & Lawrence 1993). Indeed, EPA/DHA in both muscle and eye of shrimp in the present experiment (0.59 and 0.84, respectively), were higher than the 0.28 ratio found in the diet, suggesting the importance of the correct balance between both fatty acids in this species. This hypothesis is supported by the observation that, in a closely related species, *P. monodon*, better growth was obtained when shrimp were fed both EPA and DHA at about 3 g kg^{-1} each in the diet, than the sole administration of DHA (3–9 g kg^{-1} in diet, Glencross & Smith 2001a). In marine

Table 6 Lipid content and average fatty acid composition of total lipids from eyes of shrimp cultured in either mesocosms or clear water system and fed several lipid sources ($n = 3$; % dry weight)

Fatty acid	Initial	FO mesocosms	NFO mesocosms	NFO clear water	DHA clear water	ARA clear water
14:0	0.21	0.16	0.15	0.09	0.08	0.08
15:1	0.06	0.05	0.04	0.02	0.02	n.d.
16:0aniso	0.23	0.19	0.18	0.16	0.16	0.16
16:0	4.49	4.59 a	4.38 a	3.30 b	3.08 b	3.14 b
16:1n-7	0.37	0.40 a	0.28 b	0.14 c	0.12 c	0.13 c
16:2	0.20	0.18	0.12	0.10	0.08	0.09
17:1	0.04	0.03	n.d.	n.d.	n.d.	n.d.
16:4n-1	n.d.	0.28	0.26	0.28	0.28	0.31
16:4n-3	0.03	0.03	n.d.	0.02	0.02	n.d.
18:0	1.96	1.78	1.51	1.39	1.28	1.44
18:1n-9	1.82	1.77 b	3.22 a	3.67 a	2.87 ab	3.05 a
18:1n-7	0.66	0.61 a	0.49 ab	0.41 b	0.36 b	0.38 b
18:2n-6	1.45	1.96	2.06	2.66	2.28	2.42
18:3n-6	0.04	0.04	0.03	0.02	n.d.	n.d.
18:3n-3	0.14	0.13	0.13	0.14	0.08	0.12
20:0	0.08	0.07	0.06	0.06	0.05	0.06
20:1n-9	0.24	0.23	0.22	0.19	0.19	0.19
20:1n-7	0.03	0.05	n.d.	n.d.	n.d.	n.d.
20:2n-6	0.13	0.19	0.18	0.21	0.20	0.21
20:3n-6	0.03	n.d.	n.d.	n.d.	n.d.	n.d.
20:4n-6	0.65	0.50 a	0.54 a	0.32 b	0.19 c	0.57 a
20:3n-3	0.03	0.04	0.05	0.08	0.02	0.03
20:4n-3	0.04	0.07	0.05	0.27	0.02	n.d.
20:5n-3	3.18	2.70 a	2.22 a	1.71 b	1.64 b	1.51 b
22:0	0.10	0.14	0.12	0.14	0.08	0.08
22:1n-11	n.d.	0.11	0.10	0.12	0.04	0.04
22:3n-6	0.03	0.32 b	0.67 a	0.07 c	0.11 c	n.d.
22:4n-6	n.d.	0.31	0.35	n.d.	0.04	n.d.
22:5n-6	0.04	0.45	0.16	0.11	0.17	n.d.
22:4n-3	n.d.	n.d.	n.d.	0.08	0.11	n.d.
22:5n-3	0.17	0.36 a	0.23 b	0.11 c	0.09 cd	0.05 c
24:0	0.09	0.13	n.d.	0.06	0.13	n.d.
22:6n-3	2.97	2.95 a	2.53 ab	1.73 c	1.95 b	1.62 c
24:1	n.d.	n.d.	n.d.	n.d.	0.16	n.d.
Saturated	7.15	7.01 a	6.41 b	5.16 c	4.74 c	4.96 c
Monounsaturated	3.15	3.22 b	4.33 a	4.31 a	3.52 b	3.67 b
n-3	6.56	6.25 a	5.14 b	3.91 c	3.85 c	3.31 c
n-6	2.37	3.56	3.64	3.29	2.77	3.19
n-9	2.06	2.07	3.46	3.89	3.07	3.24
n-3 HUFA	6.39	6.11 a	5.01 b	3.76 c	3.75 c	3.19 c
EPA/ARA	4.85	5.43	4.09	5.38	8.50	2.67
EPA/DHA	1.07	0.92	0.88	0.99	0.84	0.93
Crude lipids	19.5 a	20.57 a	19.95 a	16.93 b	15.26 b	15.53 b

Different letters in the same row denote significant differences ($P < 0.05$).
n.d., not detected.

organisms EPA is a main component of polar lipids regulating membrane integrity and function (Izquierdo 2005). Moderate dietary levels of this fatty acid also enhance DHA incorporation into fish larval PL (Izquierdo *et al.* 2000; Izquierdo *et al.* 2001), causing a sparing effect of such an important fatty acid.

Arachidonic acid enhances shrimp survival, but also seems to negatively affect growth. In *F. chinensis* ARA seems to be an important fatty acid but is not able to promote growth as much as DHA (Xu *et al.* 1994). Growth of *P. monodon*-fed diets containing ARA was poorer than those fed diets where it was not present (Glencross & Smith 2001b), although a lower survival was found in the absence of this fatty acid in the diet. ARA has been found to improve survival, growth, flatfish pigmentation and stress resistance in several fish species (Izquierdo *et al.* 2000; Izquierdo 2005). Although

inhibition of Δ6 desaturase activity by high dietary levels of ARA have been claimed to be one of the causes of the negative effects of this fatty acid in shrimp (Glencross & Smith 2001b), the higher 20:4n-3, 20:5n-3 and 20:3n-6 and lower 18:2n-6 and 18:3n-3 in ARA diet-fed shrimp in comparison with DHA diet-fed animals in the present study does not support this hypothesis. As found in diets enriched with DHA, shrimp-fed diets enriched in ARA in the present experiment had very low EPA contents, and hence EPA/ARA values were only about 1.6, whereas this ratio in muscle (Araujo & Lawrence 1993) and ovaries (Wouters *et al*. 2001) of wild *L. vannamei* are 2.41 and 2 respectively. EPA is a good substrate for some cyclo-oxygenases, being a precursor of some prostanoids in marine fish and also a main substrate for some lipoxygenases. Competition with ARA for these two types of enzymes enables it to be an important regulator of eicosanoid synthesis. Thus, competition of ARA with EPA for several types of enzymes, including cyclo-oxygenases and lipoxygenases eicosanoid synthesizing enzymes, could be related to the poorer general growth performance of shrimp fed the ARA diet, because EPA/ARA tissue values were 3.5 times lower than shrimp fed the DHA diet. Evidence of competition among two or more EFAs such as DHA, EPA or ARA, have been found in fish for digestive enzymes, fatty acid-binding proteins, phosphoacylglycerides synthetases, lipoxygenases and cyclo-oxygenases and probably in β-oxidation (Izquierdo *et al*. 2000, 2003; Izquierdo 2005). If such competitions among fatty acids also occur in shrimp, not only absolute dietary values for each of these EFAs but also optimum dietary ratios among them should be defined. A factorial design, such as the one proposed by Glencross & Smith (2001a) would be a better method to determine EFA requirements than the addition or deletion of single fatty acids from a standard diet as applied by many researchers. The fatty acid profile of floc in the present study in terms of EPA/DHA and EPA/ARA are between 1.3 and 3, matching better with those found in muscle of wild *L. vannamei*, around 2 (Araujo & Lawrence 1993), than that of diets containing FO. Besides, floc n-3 HUFA content was about 0.5% which is about the minimum requirement recommended for this shrimp species (González-Félix *et al*. 2004).

If LA, LNA, EPA and DHA levels supplied by protein ingredients are greater than 2.4%, 0.23%, 0.13% and 0.26% (dry weight), there maybe no need to supplement FO in diets for shrimp cultured in green water. This has been observed in other species such as carp, where the lipid requirements of fish reared in green water ponds are reduced by half of those required in intensive clear water tanks. Although complete substitution of FO by vegetable oil in diets for Pacific white shrimp reared in mesocosms system seems possible, long-term experiments including the whole grow out period should be conducted before concluding the non-essentiality of this resource under these culture conditions.

The nutritional contribution of the floc to shrimp in mesocosm culture diminishes the importance of FO as a dietary lipid source. The importance of DHA and ARA to enhancing shrimp survival in clear water conditions was also demonstrated.

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