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Influence of highly unsaturated fatty acids in live food on larviculture of mud crab *Scylla paramamosain* (Estampador 1949)

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Abstract

Two experiments were carried out to investigate the effects of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) levels in rotifers (*Brachionus plicatilis*) and *Artemia* on the survival, development and metamorphosis of mud crab *Scylla paramamosain* larvae. Five different lipid emulsions, varying in the level of total n-3 and n-6 highly unsaturated fatty acids (HUFA), DHA, EPA and ARA were used to manipulate the fatty acid profile of the live food. Fatty acid profiles of the live food and crab larvae at zoea one, three and five stages were analysed to study the HUFA uptake by the larvae. The fatty acid content of the live food affected the fatty acid profiles of the crab larvae. In both experiments, the survival rate in the zoeal stages was not statistically different among treatments. However, larval development rate and metamorphosis success were affected by the dietary treatments. In this respect, the DHA/EPA ratio in the live food seems to be a key factor. Enrichment emulsions with a very high (50%) total HUFA content but a low DHA/EPA ratio (0.6), or zero total HUFA content caused developmental retardation and/or metamorphosis failure. An emulsion with a moderate total HUFA (30%) and a high DHA/EPA ratio (4) was the best in terms of larval development during the zoeal stages and resulted in improved metamorphosis. Dietary ARA seemed to improve first metamorphosis, but its exact role needs further clarification. For the larval rearing of *S. paramamosain*, an enrichment medium containing about 30% total n-3 HUFA with a minimum DHA/EPA ratio of 1 is recommended. Further investigation is needed on the total HUFA and optimum DHA/EPA ratio requirements for each crab larval stage.

Keywords: *Scylla*, HUFA, live food, enrichment

Introduction

Johnston and Keenan (1999) listed a number of benefits of mud crab over shrimp farming such as providing more reliable income and return of initial investment resulting from higher survival due to superior adaptation to the mangrove environment, higher price per kilogram with little capital, high development rate and lower disease risk. However, mud crab farming currently relies entirely on wild seed stock and the main obstacle for the development of mud crab culture is the availability of hatchery-reared seed (Liong 1992; Keenan 1999; Mann, Asakawa, Pizzuto, Keenan & Brock 2001; Xuan 2001). Of the four *Scylla* species, *Scylla paramamosain* is dominant in Vietnam (Keenan, Davie & Mann 1998).

One important factor influencing the survival and development of marine larvae is the dietary highly unsaturated fatty acid (HUFA) composition. Sorgeloos, Dhert and Candreva (2001) reviewed the history of research on dietary HUFAs. In the 1980s, maximum attention was dedicated to the presence of eicosapentaenoic acid (20:5n-3, EPA) in *Artemia* as a

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guarantee for successful production of marine fish larvae. In the late 1980s and the early 1990s, more attention was given to the level of docosahexaenoic acid (22:6n-3, DHA) because good survival appeared to be correlated with EPA, but DHA improved larval quality and development. The importance of DHA, more particularly the requirement for high DHA/EPA ratios in promoting development, stress resistance and pigmentation, was also demonstrated (Sorgeloos *et al.* 2001). Recent work showed that arachidonic acid (20:4n-6, ARA) may also play a significant role (Castell, Bell, Tocher & Sargent 1994; Estévez, McEvoy, Bell & Sargent 1999; Koven, Barr, Lutzky, Ben-Atia, Harel, Behrens, Weiss & Tandler 2000). Arachidonic acid may improve larval development and pigmentation in several marine fish species because it provides precursors for eicosanoid production.

Supplementation of essential fatty acids through the live food, mainly rotifers (Olsen, Reitan & Vadstein 1993; Dhert, Rombaut, Suantika & Sorgeloos 2001) and *Artemia* (Watanabe 1982; Sorgeloos *et al.* 2001), has been carried out in marine fish species, e.g. seabass *Dicentrarchus labrax*, flounder *Paralichthys olivaceus*, striped jack *Pseudocaranx dentex*, gilthead seabream *Sparus aurata*, red seabream *Pagrus major*, turbot *Scophthalmus maximus* . . . (Watanabe 1993; Sargent, Bell, Bell, Henderson & Tocher 1995), shrimp species, e.g. tiger shrimp *Penaeus monodon*, *P. japonicus*, *Litopenaeus vannamei*, etc. (Rees, Curé, Piyatiratitivoraku, Sorgeloos & Menasveta 1994; Lavens & Sorgeloos 2000) and bivalves species, e.g. Manila clam *Tapes philippinarum*, Pacific blue mussel *Mytilus edulis*, oyster *Crassostrea gigas*, scallop *Placopecten magellanicus*, etc. (Coutteau, Castell, Ackman & Sorgeloos 1996; Caers, Coutteau, Lombeida & Sorgeloos 1998). Although data on the nutritional requirements of the larval stages of brachyuran crabs are rather limited, the requirement for dietary fatty acids has been demonstrated for several species, including *Scylla* spp. (Levine & Sulkin 1984; Hamasaki, Takeuchi & Sekiya 1998; Takeuchi, Satoh, Sekiya, Shimizu & Watanabe 1999; Kobayashi, Takeuchi, Arai & Sekiya 2000; Takeuchi, Kobayashi, Shimizu & Sekiya 2000; Suprayudi, Takeuchi, Hamasaki & Hirokawa 2002). Despite these findings, problems with reliable rearing techniques and the often low and inconsistent larval survival of *Scylla* larvae have hampered nutritional research considerably and contradictory results have been reported. Takeuchi *et al.* (2000), for example, showed that mud crab larvae fed rotifers and *Artemia* enriched with HUFAs exibited increased survival and performance. On the other hand, Hamasaki, Suprayudi and Takeuchi (2002) reported that elevated levels of EPA in the live food resulted in abnormal development and mortality of the larvae at metamorphosis. In a study comparing different *Artemia* strains and *Artemia* enrichment products, Mann *et al.* (2001) found no influence of the n-3 HUFA level on the ability of the larvae to complete development. Based on this, it is clear that exact dietary requirements for n-3 HUFA of mud crab larvae have not yet been established.

In order to define more clearly the importance of n-3 HUFA in the diet of *Scylla* larvae, the present study was conducted to evaluate the effect of the level and ratio of specific n-3 (DHA and EPA) and n-6 (ARA) HUFAs in live food on the fatty acid composition and culture larval performance of mud crab *S. paramamosain* during culture.

Materials and methods

Source of larvae

Females with fully matured ovaries were bought from local markets in the coastal area and transported to the hatchery of College of Aquaculture and Fisheries, Can Tho University, Vietnam, where they were reared in $30\,g\,L^{-1}$ seawater under standard conditions and induced to spawn using unilateral eyestalk ablation as described in Nghia (2004). One to 2 days before hatching, the berried female was moved to a 500 L fibreglass hatching tank containing seawater of the same salinity and connected to a biofilter. Newly hatched larvae (zoeae) in the hatching tank were selected based on their phototactic behaviour. Upon switching off aeration for 2 min, those larvae that swam actively and concentrated on the water surface under daylight were considered to be the healthiest. In order to wash out the attached waste matter and to slowly acclimate the larvae to the new rearing conditions (i.e. temperature), the larvae were placed in a 50 L plastic mesh bucket and slowly rinsed with water from the larval rearing containers for 20–30 min. Then, they were distributed evenly into the experimental containers.

Experimental design

Five different International Council for Exploration of the Sea (ICES) emulsions thereof were tested for rotifer and *Artemia* enrichment: emulsion 0/- (a coconut

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emulsion, free of HUFA, mainly consisting of saturated fatty acids); emulsion 50/0.6 (containing approximately 50% n-3 HUFA on total fatty acids with a DHA/EPA ratio of 0.6); emulsion 30/0.6 (30% n-3 HUFA with a DHA/EPA ratio of 0.6); emulsion 30/4 (30% n-3 HUFA with a DHA/EPA ratio of 4); and emulsion 30/4/ARA (a mixture of 75% of the 30/4 emulsion and 25% of an emulsion containing 40% ARA) (Table 2).

In experiment 1, the same enrichment emulsions were used for both rotifers and *Artemia* within one treatment. In experiment 2, one treatment was included (30/0.6–30/4) where rotifers were enriched with the 30/0.6 emulsion and *Artemia* with the 30/4 emulsion in addition to other treatments using the same emulsion for enriching both rotifers and *Artemia*. For each experiment, a control treatment was included (see 'Live food culture, enrichment and feeding to crab larvae' and Table 2). Where appropriate, the control treatments are clarified as 'unenrich' control and 'Culture Selco' control for the control treatments of experiments 1 and 2 respectively.

Larval rearing

Larval rearing procedures

In both experiments, a small-scale batch rearing system consisting of 1 L acrylic bowls was used. The bowls were randomly distributed over a heated water bath to provide identical rearing conditions. Rearing water ($30 \pm 1\,g\,L^{-1}$ salinity and 30 ± 1 °C) was diluted from brine ($90–110\,g\,L^{-1}$) with tap water and chlorinated before use. Gentle aeration was applied to all bowls. Each day, the remaining larvae were pipetted into new bowls containing fresh seawater. These new bowls were incubated beforehand in the same water bath to equilibrate temperature. In order to exclude bacterial interference, $10\,mg\,L^{-1}$ oxytetracycline was applied to the bowls daily. In experiment 1, each treatment was also repeated in two 100 L cylindro-conical fibreglass tanks connected to a submerged biological filter and operated in the recirculation mode. An upwelling system with a water renewal rate of 100% every 3–4 h was used. Water was evacuated at the water surface through a 70 or 300 μm filter screen during the rotifer and *Artemia* feeding stages, respectively, to retain larvae and live food in the culture tank. Gentle aeration was applied to all rearing tanks. Formalin at a concentration of $20\,\mu L\,L^{-1}$ was applied to the whole system every 2 days to prevent or reduce fungal and bacterial development.

Zoea 1 (Z1) were reared until the megalopa and first crab stage. In experiment 1, megalopae were separated daily from the rearing containers and redistributed by treatment in 1 L bowls at a density of 6–8 megalopae $bowl^{-1}$. Polyvinyl chloride sponges ($1\,cm^3$ pieces) were provided as shelters in each bowl to prevent cannibalism. In experiment 2, megalopae were separated from the cultures several times per day and reared individually to crab stage in 100 mL cups without aeration. The larval rearing conditions for all experiments are summarized in Table 1.

Live food culture, enrichment and feeding to crab larvae

The same rotifer strain, *Brachionus plicatilis* L-strain with a lorica length and width of 164 ± 22 and 120 ± 22 μm, respectively, was used in all the experiments. In experiment 1, rotifers were cultured on baker's yeast in an indoor recirculating system using 100 L fibreglass tanks (Suantika 2001). The water recirculation rate in this system was 100% day^{-1}. In experiment 2, rotifers were cultured outdoors in an integrated recirculating system. The system consisted of two $10\,m^3$ tanks inoculated with *Chlorella* spp. and stocked with Tilapia ($1\,kg\,m^{-3}$), connected to a $4\,m^3$ fibreglass rotifer culture tank. When the algal concentration reached 10 million cells mL^{-1}, rotifers were stocked at 100 individuals mL^{-1} and the system was recirculated (100% change every 4 h). Starting from the third day, part of the rotifer population was then harvested daily as feed for the crab larvae.

Table 1 Summary of larval rearing conditions and water quality parameters in the two experiments

Experiment	Culture period (days)	Stocking density ($Z1\,L^{-1}$)	Container		Water exchange	Water quality parameters			
			Volume (L)	Type		Temp. (°C)	Salinity ($g\,L^{-1}$)	NH_4^+ ($mg\,L^{-1}$)	NO_2 ($mg\,L^{-1}$)
1	29	50	1	Bowl	Batch	30.0 ± 1.0	30.0 ± 1.0	< 1.0	<0.3
	15	50	100	Tank	Re-circulation				
2	31	100	1	Bowl	Batch				

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Table 2 Summary of the treatments used in the two experiments

Experiment	Treatment							Number of replicates
	Control	0/-	50/0.6	30/0.6	30/4	30/0.6–30/4	30/4/ARA	
1	Unenriched rotifers and *Artemia*	Rotifers and *Artemia* enriched with emulsion 0/-	Rotifers and *Artemia* enriched with emulsion 50/0.6	Rotifers and *Artemia* enriched with emulsion 30/0.6	Rotifers and *Artemia* enriched with emulsion 30/4		Rotifers and *Artemia* enriched with emulsion 30/4/ARA	Four bowls for experiment and two tanks for HUFA sampling
2	Rotifers and *Artemia* enriched with Culture Selco (emulsion 25/1)			Rotifers and *Artemia* enriched with emulsion 30/0.6	Rotifers and *Artemia* enriched with emulsion 30/4	Rotifers and *Artemia* enriched with emulsion 30/0.6 and 30/4 respectively		Three bowls

Rotifers were fed to the crab larvae from 0 to 6 days after hatch (DAH) and *Artemia* replaced rotifers from the evening of DAH 6 on onset of Z3 stage.
HUFA, highly unsaturated fatty acids; ARA, arachidonic acid.

Artemia (Vinh Chau strain) cysts were disinfected with chlorine, incubated and hatched following standard methods (Sorgeloos, Lavens, Léger, Tackaert & Versichele 1986). Rotifers and *Artemia* were enriched with different ICES standard reference emulsions. Rotifer enrichment was performed at a density of 500 ind mL^{-1}, using two separate doses of 0.125 g L^{-1} at a 3-h interval. The temperature and salinity of the water was maintained at 25–30 °C and 25–30 g L^{-1} respectively. For *Artemia* enrichment, newly hatched *Artemia* were concentrated to 200 ind mL^{-1} and the oil emulsions were used at two separate doses of 0.3 g L^{-1} at a 12 h interval. Temperature and salinity were kept at 30 °C and 30 g L^{-1} respectively.

In experiment 1, control *Artemia* were not enriched at 30 °C for 24 h. In experiment 2, the control rotifers and *Artemia* were enriched with the commercial product Culture Selco® (28% n-3 HUFA with a DHA/EPA ratio of 1; INVE Aquaculture NV, Belgium) under similar conditions (live food density, salinity and temperature, enrichment dose and time) as for the ICES emulsions.

Larvae were fed rotifers from 0 to 6 days after hatch (Z1 and Z2) at 45 ind. mL^{-1} and then *Artemia* thereafter at 7–10 ind. mL^{-1} daily. In bowls, preys were replaced by new ones upon changing water daily. In tanks, old preys were flushed out through a larger-sized net until 1–2 ind. mL^{-1} left, and then new ones were added.

Evaluation criteria

Fatty acid composition

In experiment 1, the ICES emulsions, the different enriched live food (rotifers and *Artemia*) and the crab larvae (Z1, Z3 and Z5) were sampled for fatty acid analysis. Samples were washed with freshwater and stored at −80 °C until fatty acid methyl esters (FAME) analysis. Fatty acid methyl esters analysis were prepared by direct transesterification with 10% acetyl chloride in methanol (Lepage & Roy 1984) and analysed in a Chrompack CP9001 gas chromatograph (Sigma-Aldrich, St Louis, MO, USA) equipped with an autosampler. Injection was performed on a column into a very polar 50 m capillary column, BPX70, with a diameter of 0.32 mm and a layer thickness of 25 μm connected to a 2.5 m methyl deactivated pre-column. The carrier gas was H_2 and the detection mode was FID. The oven temperature was set to increase the initial temperature from 85 to 150 °C at a rate of 20 °C min^{-1}, from 150 to 152 °C at 0.1 °C min^{-1}, from 125 to 174 °C at 0.7 °C min^{-1}, from 174 to 180 °C at 10 °C min^{-1} and to remain at 180 °C for 2 min. Identification was based on standard reference mixtures (Nu-Check-Prep, Elysian, MN, USA). Integrations and calculations were performed with the software program 'Maestro' (Chrompack). The method consists of a direct acid-catalysed transesterification of dry samples (10–150 mg) without prior lipid extraction. Only the most important individual essential fatty acids and groups (ARA, EPA, DHA,

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total n-3, total n-6 and total HUFA) and ratios thereof (DHA/EPA, ARA/EPA and Σn-3/Σn-6) are reported. Total HUFA was defined as all fatty acids with more than 20 carbon atoms and three or more unsaturated carboxyl bonds, and total n-3 HUFA as all n-3 fatty acids ≥ 20:3n-3.

Larval performance

The average survival rates were calculated by individually counting all surviving larvae in each replicate. In experiment 1, zoeal development was monitored every 3 days. Ten larvae were sampled from each 100 L tank. Larvae were examined under a dissecting microscope and a corresponding value was assigned to each stage as follows: Z1 = 1; Z2 = 2, etc. A larval stage index (LSI) was then calculated as the average stage of the 10 larvae. To compare the larval development in each treatment, an average LSI was calculated from the average LSI value of the replicate tanks of the same treatment. In experiment 2, larvae were staged visually upon counting the surviving larvae daily.

Two parameters were used to evaluate the success of metamorphosis. First is the determination of the metamorphosis rate (% MR) or the percentage survival through metamorphosis: (i) percentage of Z5 that survived to megalopa (MR1) and (ii) percentage of megalopa that survived to the crab stage (MR2). Second is the determination of the average time needed for the larvae to go through metamorphosis and the total duration (minimum and maximum) of the first and second metamorphoses. In addition, the overall cumulative survival rates of Z1 to megalopa and crab stage were graphically presented based on the pooled data of all replicates in each treatment for experiment 2.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the data. The Tukey HSD *post-hoc* analysis was used to detect differences between means. Data were transformed using the arcsine-square root (for percentage, i.e. survival rate) or logarithmic transformations (for other parameters) where necessary (Sokal & Rohlf 1995). The two-tailed Fisher exact test (modified from the contingency table method) was used to compare the ratios (expressed in per cent) of treatments with pooled data. Pearson's coefficient was used to examine the correlation between the fatty acid composition of the live food and the crab larvae, and the correlation between the fatty acid composition of the live food and larval development (i.e. LSI values and metamorphosis rates). P was set at 0.05. Significant differences at 0.01 were also mentioned. All analyses were performed using the statistical program STATISTICA 6.0.

Results

Fatty acid composition of live food and crab larvae (experiment 1)

The composition of the emulsions (the same batch was used for both experiments) is presented in Table 3. The fatty acid profile of the live food (Table 4) and the larvae (Table 5) was determined only in

Table 3 Fatty acid composition of the ICES emulsions used to enrich the live food in the two experiments

ICES emulsion fatty acid content	0/-	50/0.6	30/0.6	30/4	30/4/ARA
ARA (mg g^{-1})	0.00	10.40	5.25	4.71	94.51
EPA (mg g^{-1})	0.00	261.40	103.70	42.13	31.58
DHA (mg g^{-1})	0.00	163.60	63.73	161.87	121.46
Σn-3* (mg g^{-1})	0.00	499.80	167.43	204.00	156.19
Σn-6† (mg g^{-1})	2.88	59.50	36.57	50.91	159.93
ΣHUFA‡ (mg g^{-1})	0.00	524.40	172.68	208.72	251.17
DHA/EPA	–	0.63	0.61	3.84	3.85
ARA/EPA	–	0.04	0.05	0.11	2.99
Σn-3/Σn-6	0.00	8.40	4.58	4.01	0.98
Σn-3/FAME§ (%)	0.00	51.41	29.29	30.48	22.34

*Total n-3 HUFA.
†Total n-6 HUFA.
‡Total HUFA.
§Fatty acid methyl esters = total fatty acid content.
ICES, International Council for Exploration of the Sea; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HUFA, highly unsaturated fatty acids.

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Table 4 Fatty acid composition of rotifers and *Artemia* used in experiment 1

	Rotifers						*Artemia*					
Fatty acid content	**CNL**	**0/-**	**50/0.6**	**30/0.6**	**30/4**	**30/4/ARA**	**CNL**	**0/-**	**50/0.6**	**30/0.6**	**30/4**	**30/4/ARA**
ARA (mg g^{-1})	1.08	0.75	2.15	1.65	1.03	7.13	3.86	4.16	6.50	2.50	8.34	25.77
EPA (mg g^{-1})	2.33	2.65	28.61	18.91	7.19	11.26	8.94	10.49	40.09	29.60	23.29	27.49
DHA (mg g^{-1})	1.94	2.10	19.99	13.34	18.45	23.61	0.35	0.21	19.53	11.20	20.61	19.07
Σn-3* (mg g^{-1})	4.27	4.76	48.60	32.25	25.64	34.88	9.49	11.05	64.99	45.10	46.64	48.79
Σn-6† (mg g^{-1})	7.05	14.96	17.17	12.38	11.36	23.15	7.67	14.56	16.49	14.10	22.82	47.72
ΣHUFA‡ (mg g^{-1})	5.35	5.51	50.75	33.90	26.67	42.01	14.00	16.02	73.51	50.60	58.00	77.96
DHA/EPA	0.84	0.79	0.70	0.71	2.57	2.10	0.04	0.02	0.49	0.38	0.88	0.69
ARA/EPA	0.46	0.28	0.08	0.09	0.14	0.63	0.43	0.40	0.16	0.08	0.36	0.94
Σn-3/Σn-6	0.61	0.32	2.83	2.61	2.26	1.51	1.24	0.76	3.94	3.20	2.04	1.02

*Total n-3 HUFA.
†Total n-6 HUFA.
‡Total HUFA.
ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HUFA, highly unsaturated fatty acids; CNL, Control.

Table 5 Fatty acid composition of *Scylla paramamosain* zoea 1 (newly hatched zoea), zoea 3 and zoea 5 fed different enriched live food in experiment 1

		Zoea 3						Zoea 5					
Fatty acid content	**Zoea 1**	**CNL**	**0/-**	**50/0.6**	**30/0.6**	**30/4**	**30/4/ARA**	**CNT**	**0/-**	**50/0.6**	**30/0.6**	**30/4**	**30/4/ARA**
ARA (mg g^{-1})	6.59	4.56	2.73	4.03	1.84	2.27	4.93	3.77	4.74	4.41	3.70	4.73	6.76
EPA (mg g^{-1})	12.13	7.43	7.29	18.78	8.76	6.33	6.84	8.17	10.92	16.79	12.12	11.44	12.66
DHA (mg g^{-1})	9.70	4.03	4.32	13.07	6.77	8.20	8.00	0.87	1.78	6.21	4.34	6.78	6.84
Σn-3* (mg g^{-1})	21.83	11.46	11.61	31.85	15.53	14.53	14.84	9.04	13.25	23.00	16.46	19.07	19.50
Σn-6† (mg g^{-1})	11.47	15.50	12.59	14.22	6.20	5.54	11.00	6.10	15.54	9.86	7.89	11.55	12.80
ΣHUFA‡ (mg g^{-1})	28.42	16.02	14.33	35.88	17.36	16.79	19.77	12.81	19.18	27.41	20.15	24.88	26.25
DHA/EPA	0.80	0.54	0.59	0.70	0.77	1.29	1.17	0.11	0.16	0.37	0.36	0.59	0.54
ARA/EPA	0.54	0.61	0.37	0.21	0.21	0.36	0.72	0.46	0.43	0.26	0.30	0.41	0.53
Σn-3/Σn-6	1.90	0.74	0.92	2.24	2.50	2.62	1.35	1.48	0.85	2.33	2.09	1.65	1.52

*Total n-3 HUFA.
†Total n-6 HUFA.
‡Total HUFA.
ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HUFA, highly unsaturated fatty acids; CNT, Control.

experiment 1. We assume that, except for the control treatments (which differed between the experiments), similar live food enrichment levels were obtained in both experiments. Rotifer composition was clearly influenced by the enrichment treatments. The total n-3 content of the rotifers increased from 4 mg g^{-1} in the control to 26–35 mg g^{-1} in treatments 30/0.6, 30/4 and 30/4/ARA. The highest n-3 level (49 mg g^{-1}) was obtained in treatment 50/0.6. Treatment 0/- resulted in a level similar to the control. As expected, treatment 50/0.6 resulted in the highest EPA level in the rotifers (29 mg g^{-1}). The DHA/EPA ratio of the rotifers was the highest for treatments 30/4 and 30/4/ARA (2.57 and 2.1 respectively), compared with 0.71–0.84 for the other treatments. Treatment 30/4/ARA resulted in elevated ARA and total n-6 levels and hence a high ARA/EPA ratio. The control and 0/- rotifers were especially low in ARA, EPA and DHA. Similar patterns were observed for *Artemia*. However, using the same enrichment medium, DHA levels and the DHA/EPA ratio were lower in *Artemia* compared with rotifers, except for treatment 30/4. The DHA level in the control *Artemia* was extremely low, resulting in a very low DHA/EPA ratio. However, absolute levels of most other fatty acids were higher in *Artemia*. Compared with unenriched rotifers, the control *Artemia* also contained relatively high amounts of EPA, total n-3 and ARA. Overall, the rotifer and *Artemia* composition reflected very well the total n-3, EPA, DHA and ARA levels of

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Table 6 Pearson correlation coefficients (r^2) between the fatty acid composition of the live food and those of the crab larvae in experiment 1

Fatty acid Correlation	ARA	EPA	DHA	Σn-3†	DHA/EPA	ARA/EPA	Σn-3/Σn-‡
Rotifers – Zoea 3	0.36	0.72*	0.62	0.65	0.90**	0.96**	0.86**
Artemia – Zoea 5	0.91**	0.83*	0.98**	0.91**	0.97**	0.81*	0.86**

*Significant correlations ($P<0.05$).
**Significant correlations ($P<0.01$).
†Total n-3 HUFA.
‡Total n-6 HUFA.
ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HUFA, highly unsaturated fatty acids.

Table 7 Mean survival rates and larval stage index (LSI) values (± standard deviation) of *Scylla paramamosain* larvae fed different enriched rotifers and *Artemia* in experiment 1

Treatment	Days after hatch				
	3	6	9	12	15
Survival rates* (%)					
Control	96 ± 2^a	92 ± 5^a	88 ± 4^a	64 ± 5^a	28 ± 11^a
0/-	94 ± 5^a	89 ± 8^a	83 ± 10^a	59 ± 7^a	34 ± 7^a
50/0.6	95 ± 4^a	92 ± 4^a	85 ± 8^a	63 ± 15^a	35 ± 14^a
30/0.6	93 ± 3^a	86 ± 5^a	80 ± 4^a	67 ± 7^a	43 ± 6^a
30/4	96 ± 4^a	87 ± 5^a	85 ± 6^a	56 ± 17^a	20 ± 10^a
30/4/ARA	96 ± 3^a	89 ± 6^a	81 ± 3^a	65 ± 13^a	41 ± 11^a
LSI*					
Control	1.7 ± 0.1^b	2.2 ± 0.1^b	3.0 ± 0.1^c	3.5 ± 0.1^c	4.4 ± 0.1^c
0/-	1.6 ± 0.0^c	2.0 ± 0.1^b	2.9 ± 0.1^c	3.4 ± 0.1^c	4.4 ± 0.1^c
50/0.6	1.7 ± 0.0^b	2.2 ± 0.1^b	3.3 ± 0.1^{ab}	3.8 ± 0.1^{ab}	4.7 ± 0.1^{ab}
30/0.6	1.6 ± 0.1^{bc}	2.2 ± 0.1^b	3.2 ± 0.1^b	3.7 ± 0.1^b	4.6 ± 0.1^b
30/4	1.9 ± 0.0^a	2.6 ± 0.2^a	3.4 ± 0.1^{ab}	3.9 ± 0.1^a	4.8 ± 0.1^a
30/4/ARA	1.9 ± 0.0^a	2.5 ± 0.1^a	3.3 ± 0.1^a	3.8 ± 0.1^{ab}	4.8 ± 0.3^{ab}

*Survival rates or LSI values in the same column followed by the same superscript letter are not statistically different ($P \geq 0.05$). For treatment descriptions refer to Tables 2 and 3.
ARA, arachidonic acid.

the emulsions and thus the theoretic design of the experiments.

The dietary fatty acid level affected the composition of the larvae (Table 5). Treatment 50/0.6 had the highest total n-3 and EPA levels in Z3 and Z5, while intermediate results were obtained for treatments 30/0.6, 30/4 and 30/4/ARA. The DHA level and DHA/EPA ratio increased in all treatments, except 0/-, but was the highest for 30/4 and 30/4/ARA. A very low DHA/EPA ratio was observed for Z5 in the control and 0/- treatments (0.11 and 0.16 respectively). The ARA content of Z3 and Z5 in the 30/4/ARA treatment (5 and 7 mg g^{-1} respectively) was higher than those in the other treatments. Treatment 30/4/ARA also resulted in the highest ARA/EPA ratio in the crab larvae.

The correlation coefficients between specific fatty acid levels in the crab larvae and live food are summarized in Table 6. For Z3, the EPA level and the DHA/EPA, ARA/EPA and n-3/n-6 ratios were significantly correlated to those of the rotifers (all at $P<0.01$). No significant correlation was found for the level of ARA, DHA and total n-3 ($r^2 = 0.36$, 0.62 and 0.65 respectively). The fatty acid levels and ratios were significantly correlated to those of the *Artemia* in Z5 ($P<0.01$, except the ARA/EPA ratio at $P<0.05$).

Larval survival

Survival generally decreased in later larval stages and high variability was observed between replicates (Tables 7 and 8). This made comparison difficult and hence not many significant differences in survival were observed between any of the treatments. In

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Table 9 Mean survival from zoea 5 to megalopa (MR1) and megalopa to crab (MR2); and mean ± standard deviation (minimum and maximum) time (days after hatch) from Z1 to reach first and second metamorphosis of *Scylla paramamosain* larvae fed different enriched rotifers and *Artemia* in experiment 1

Treatment	Survival* (%)		Metamorphosis time† (days after hatch)	
	MR1	MR2	To megalopa	To crab 1
Control	31[b]	13[b]	19.3 ± 1.6[a] (18–22)	27.0 ± 1.4[ab] (26–28)
0/-	32[b]	11[b]	18.2 ± 1.5[ab] (17–22)	27.5 ± 2.1[ab] (26–29)
50/0.6	30[b]	73[a]	18.3 ± 1.5[ab] (16–23)	27.0 ± 1.6[a] (24–29)
30/0.6	34[b]	73[a]	17.1 ± 1.0[b] (15–19)	25.9 ± 1.2[ab] (24–28)
30/4	30[b]	92[a]	17.8 ± 1.6[ab] (16–21)	24.3 ± 1.4[b] (22–27)
30/4/ARA	50[a]	93[a]	17.3 ± 1.4[b] (15–21)	24.8 ± 1.4[b] (22–27)

*No standard deviation available due to very low survival in some replicates, two-tailed Fisher exact test was used to compare survival ratios (expressed in %) of treatments with pooled data of replicates in each trearment.
†Values in the same column followed by the same superscript letter are not statistically different ($P \geq 0.05$). For treatment names refer to Tables 2 and 3.
ARA, arachidonic acid.

Table 10 Mean ± standard deviation survival from zoea 5 to megalopa (MR1) and megalopa to crab (MR2); and mean ± standard deviation time (days after hatch) from Z1 to reach first and second metamorphosis of *Scylla paramamosain* larvae fed different enriched rotifers and *Artemia* in experiment 2

Treatment	Survival* (%)		Metamorphosis time* (days after hatch)	
	MR1	MR2	To megalopa	To crab 1
Control	48 ± 9[a]	67 ± 20[a]	19.3 ± 1.7[ab]	25.7 ± 2.3[b]
30/0.6	65 ± 14[a]	78 ± 24[a]	19.4 ± 2.1[ab]	26.6 ± 2.1[ab]
30/0.6–30/4	50 ± 16[a]	67 ± 20[a]	20.1 ± 2.5[a]	27.1 ± 2.0[a]
30/4	66 ± 20[a]	81 ± 3[a]	18.6 ± 1.5[b]	26.5 ± 2.0[ab]

*Values in the same column followed by the same superscript letter are not statistically different ($P \geq 0.05$). For treatment names refer to Tables 2 and 3.

Experiment 2

No statistical differences were found among the treatments for MR1 and MR2 (Table 10 and Fig. 1). However, Fig. 1 is based on pooled data of replicates of each treatment instead of means of treatments as in Table 10. As a result, the data of treatments showed similarity or slight difference between two statistical methods. The data from figure were considered to be more realistic than that of the table because the latter were affected by high values of standard deviation. The mean first metamorphosis time in treatment 30/4 was significantly shorter (± 18 days) compared with treatment 30/0.6–30/4 (± 20 days) ($P<0.01$), while those of the 'Culture Selco' control and 30/0.6 were intermediate (± 19 days) ($P<0.01$). The average time needed to complete the second metamorphosis was significantly the longest for treatment 30/0.6–30/4 (± 27 days) and the shortest in the control (± 25 days) ($P<0.01$). Treatments 30/0.6 and 30/4 had intermediate values (± 26 days).

The cumulative metamorphosis rates from Z1 to megalopa and crab of the different treatments are presented in Fig. 2. From this, it is obvious that treatment 30/4 outperformed all other treatments ($P<0.01$). The 'Culture Selco' control was not different from treatment 30/0.6–30/4, but significantly better than 30/0.6 ($P<0.05$ and 0.01 for MR1 and MR2 respectively).

Correlation between the fatty acid composition of the live food and larval development rate and metamorphosis success

On DAH 3 and 6, there was a significant correlation ($P<0.01$) between the LSI values and the rotifer DHA/EPA ratios ($r^2=0.86$ and 0.87 respectively;

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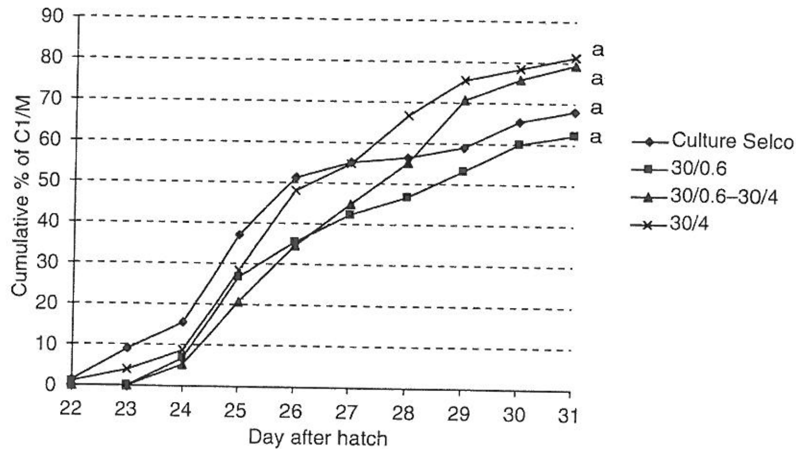

Figure 1 Cumulative metamorphosis rates from megalopa to first crabs of *Scylla paramamosain* larvae fed different enriched live food in experiment 2. For treatment descriptions, refer to Tables 2 and 3. Curves for each survival followed by the same letter are not statistically different ($P \geq 0.05$).

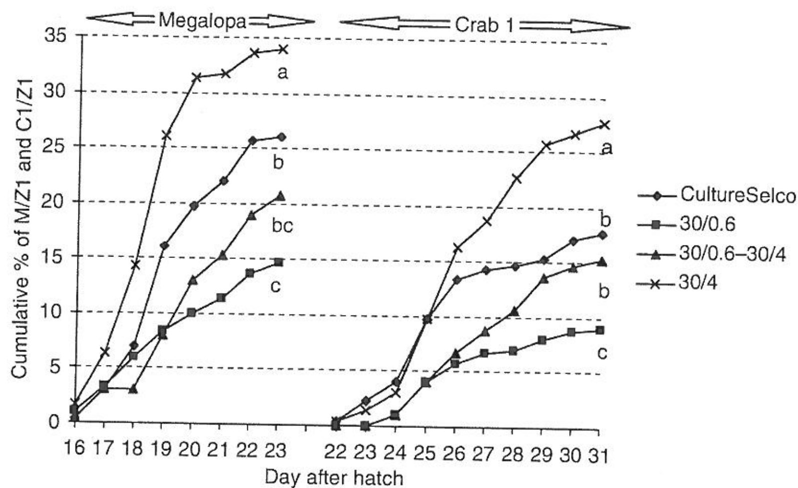

Figure 2 Cumulative metamorphosis rates from zoea 1 to megalopa and first crabs of *Scylla paramamosain* larvae fed different enriched live food in experiment 2. For treatment descriptions, refer to Tables 2 and 3. Curves for each survival followed by the same letter are not statistically different ($P \geq 0.05$).

Table 11). This shows that the DHA/EPA ratio was the most important factor affecting development in the early stages.

From DAH 9–15, there were significant correlations (r^2 ranging from 0.77 to 0.98) between the LSI values of crab larvae and the DHA content ($P < 0.01$), total n-3 content ($P < 0.05$) and the DHA/EPA ratio ($P < 0.01$) of the *Artemia*. This proves that besides the DHA/EPA ratio, the absolute DHA and total n-3 content become more important for the crab larvae in the *Artemia*-feeding stage.

Neither the ARA and EPA content, nor any of the other fatty acid ratios (ARA/EPA and Σn-3/Σn-6) of the live food were correlated with the LSI values of the crab larvae. Only the ARA content and the ARA/EPA ratio of the *Artemia* correlated significantly with the MR1 ($P < 0.05$). The DHA and total n-3 content, and the DHA/EPA ratio of the *Artemia* (r^2 from 0.8 to

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Table 11 Pearson correlation coefficients (r^2) of the fatty acid composition of the live food (rotifers and *Artemia*) with larval stage index (LSI) values and first (MR1) and second (MR2) metamorphosis rates (%) in experiment 1

Fatty acid Correlation	ARA	EPA	DHA	Σn-3†	DHA/EPA	ARA/EPA	Σn-3/Σn-6‡
Rotifers–LSI DAH 3	0.30	0.00	0.52	0.13	0.86**	0.10	0.08
Rotifers–LSI DAH 6	0.18	0.00	0.56	0.17	0.87**	0.01	0.19
Artemia–LSI DAH 9	0.20	0.58	0.97**	0.79*	0.94**	0.00	0.24
Artemia–LSI DAH 12	0.28	0.60	0.98**	0.81*	0.94**	0.02	0.21
Artemia–LSI DAH 15	0.32	0.57	0.95**	0.77*	0.93**	0.03	0.15
Artemia – MR1	0.83*	0.02	0.09	0.04	0.09	0.69*	0.15
Artemia – MR2	0.30	0.61	0.92**	0.80*	0.90**	0.02	0.20

*Significant correlations at $P<0.05$.
**Significant correlations at $P<0.01$.
†Total n-3 HUFA.
‡Total n-6 HUFA.
DAH, days after hatch; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HUFA, highly unsaturated fatty acids.

0.92) correlated significantly with the MR2 values. All correlations were significant at $P<0.01$, except at $P<0.05$ for total n-3.

Discussion

Fatty acid composition of live feed and crab larvae

The ICES emulsions significantly influenced the ARA, EPA, DHA and total n-3 contents, and thus the related DHA/EPA, ARA/EPA andΣn-3/Σn-6 ratios of the live food. In general, the elevated HUFA content in the enriched rotifers and *Artemia* resulted in a significant increase in these fatty acids in the crab larvae. This confirms the finding of previous studies that dietary HUFAs are readily assimilated by *Scylla* larvae (Levine & Sulkin 1984; Hamasaki *et al.* 1998; Takeuchi *et al.* 1999, 2000; Kobayashi *et al.* 2000; Mann *et al.* 2001; Suprayudi *et al.* 2002; Davis 2003). Except for the ARA/EPA ratio, for most fatty acids, however, a better correlation was found between the composition of *Artemia* and Z5 compared with rotifers and Z3 (i.e. the ARA, DHA and total n-3 contents were not significantly correlated for the latter). This probably indirectly results from the different digestion and assimilation of HUFA in rotifers and *Artemia*. For example, it has been well known that *Artemia* catabolize DHA much more than do rotifers and thus the former are considered to be much more difficult to 'enrich' with this fatty acid (Dhert, Sorgeloos & Devresse 1993; Wouters, Van Hauwaer, Naessens, Ramos, Pedrazzoli & Lavens 1997; Navarro, Henderson, McEvoy, Bell & Amat 1999; Bell, McEvoy, Estevez, Shields & Sargent 2001). These lower levels in *Artemia* may indirectly increase retention of these fatty acids by the crab larvae.

Survival in the zoeal stages

In experiment 1, live food treatments that resulted in a low total n-3 content (4–5 and 9–11 mg g^{-1} in rotifers and *Artemia*, in the 'unenrich' control and treatment 0/- respectively) resulted in comparable survival rates compared with those treatments that gave high total n-3 contents (26–49 and 45–65 mg g^{-1} in rotifers and *Artemia* respectively). Similarly, Davis, Churchill, Hecht and Sorgeloos (2004) found that for *Scylla serrata*, survival through the first metamorphosis was not significantly different among treatments using live food that contained a wide range of total n-3 HUFA (9–18 and 2–54 mg g^{-1} in rotifers and *Artemia* respectively). Mann *et al.* (2001) also found that feeding *S. serrata* larvae with *Artemia* nauplii enriched with a commercial lipid booster did not significantly affect larval survival when the total n-3 level of the live food in the different treatments varied from 3 to 84 mg g^{-1}. In our study, the total n-3 level of rotifers in the 'starvation' control and treatment 0/- was very low. Nevertheless, Z3 in both treatments could still accumulate n-3 HUFAs to obtain a level of 11–12 mg g^{-1}, which was only slightly lower than in the other treatments (15–16 mg g^{-1}, except for the high level of 32 mg g^{-1} in treatment 50/0.6). Apparently, the high initial total n-3 content in Z1 (22 mg g^{-1}), together with the trace amounts derived from the diet, were sufficient to

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overcome deficiencies and maintain high survival up to the last zoeal stage. For the red frog crab *Ranina ranina*, it was found that dietary energy is utilized for survival first, moulting second and morphogenesis last (Minagawa 1992). Similarly, in fish, the effects of feeding HUFA-deficient and HUFA-enriched *Artemia* nauplii are not always apparent in survival (Ashraf 1993).

In the *Artemia* feeding stage, however, Z5 larvae of the 'unenrich' control and treatment 0/-, could not maintain their total n-3 content (9 and 13 mg g^{-1} respectively) above that of the *Artemia* they were fed with (9 and 11 mg g^{-1} respectively). This means that, in contrast to the rotifer feeding stage, the larvae fed with HUFA-deficient *Artemia* could not accumulate these fatty acids. Nevertheless, the survival rates in these later stages were still not significantly different between treatments on most sampling days.

Larval development

The larval development rate expressed as LSI was more affected by the dietary treatments than the survival rate. Anger, Dawirs, Anger and Costlow (1981) noticed that in brachyuran crabs, nutrition influences development more directly than survival that is affected by a variety of other factors.

In both experiments, treatment 30/4 resulted in the highest larval development rate among treatments. Enrichment media with a similar or higher total n-3 content, but lower DHA/EPA ratio (treatments 30/0.6 and 50/0.6) usually performed significantly less. This shows that high levels of HUFAs (particularly EPA as in treatment 50/0.6) are neither needed nor beneficial for the larval performance. High HUFA levels in crustacean larvae do not necessarily result in improved performance (González-Félix, Gatlin, Lawrence & Perez-Velazquez 2002), and the performance of *S. serrata* can even be compromised when HUFA is supplied at excessive levels (Suprayudi *et al.* 2002). In this study, an n-3 HUFA level of 30% in the emulsions proved to be sufficient for live food enrichment.

One of the suggested causes for the discrepancy in mud crab larval nutrition studies has been the variability in quality between different batches of larvae as in *S. paramamosain* (Zeng & Li 1999; Djunaidah, Wille, Kontara & Sorgeloos 2003) and in *S. serrata* (Mann, Asakawa & Blackshaw 1999; Millamena & Bancaya 2001; Davis 2003). Larval quality has been linked to the HUFA content (particular EPA and total n-3) as was illustrated by Churchill (2003) in *S. serrata* larvae. Furthermore, Davis (2003) showed that larvae containing different fatty acid profiles at hatch may require different levels or ratios of certain essential fatty acids in the diet. Larval quality may in turn be a result of differences in egg quality relating to different HUFA reserves from different batches that have been found commonly in the wild-caught spawners (Churchill 2003; Djunaidah *et al.* 2003). Although the selected broodstock crabs were fed the same diet, they spent different durations of time in captivity. Hence, the nutritional status of female mud crabs varies and consequently may affect the quality of eggs and Z1 larvae (Davis 2003).

Treatment 0/-, using a HUFA-deficient emulsion, produced low LSI values in both experiments throughout the rearing period. This shows that live food enrichment does not improve larval development by merely supplying extra energy. As the control treatment differed from one experiment to another, the LSI values of the control treatments in the two experiments were also different. In experiment 1, LSI values in the 'unenrich' control were always lower than those of the 'HUFA-rich' treatments. In this trial, control rotifers were grown on baker's yeast and control *Artemia* were not enriched, and hence deficient in HUFA. In experiment 2, control live food were enriched using the commercial product Culture Selco, which contains considerable amounts of n-3 HUFA. The LSI values of this 'Culture Selco' control treatment were always higher than those of 30/0.6. In the early larval stages, the 'Culture Selco' control also outperformed treatment 30/0.6–30/4; however, LSI values became similar towards the end of the rearing period. As the 'Culture Selco' control, which only has a DHA/EPA ratio of 1, resulted in similar LSI values (and survival rates) compared with treatment 30/4, it seems that an emulsion with a total HUFA content of approximately 30% with a DHA/EPA value of 1 is sufficient to satisfy the requirements for larval development in the early zoeal stages as the effects of dietary HUFAs would manifest in the metamorphosis stages.

The importance of DHA and its ratio to EPA for larval development was also obvious from the correlation coefficients between the LSI and the fatty acid profile of the live food. The LSI values on DAH 3 and 6 were only significantly correlated with the DHA/EPA level of rotifers, and not with the DHA or the total n-3 level. In contrast, during the *Artemia* feeding stage, the absolute DHA level and total n-3 level also significantly influenced larval development. This difference could be due to the difference in enrichment

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kinetics between rotifers and *Artemia*. *Artemia* is known to selectively catabolize DHA during enrichment, resulting in relatively lower total DHA levels (Dhert *et al.* 1993; Wouters *et al.* 1997; Navarro *et al.* 1999; Bell *et al.* 2001). This could explain why the absolute DHA level (and hence total n-3 level) also becomes more important during *Artemia* feeding.

Metamorphosis

Metamorphosis rate

In experiment 1, MR1 of the ARA treatment was significantly higher than in the 'n-3 HUFA treatments'. The ARA content and ARA/EPA ratio of *Artemia* also correlated significantly with the MR1 values. The role of ARA in first metamorphosis remains unclear and requires further investigation. Koven *et al.* (2000) suggested that besides DHA, not only are HUFA of the n-3 series important but that also ARA may play a significant role for the gilthead seabream (*S. aurata*) larvae. Arachidonic acid may improve larval development and pigmentation in several marine fish species because it provides precursors for eicosanoid production (Castell *et al.* 1994; Estévez *et al.* 1999). Turbot fed ARA as the only HUFA yielded higher development and survival compared with any of the DHA/ARA mixtures or DHA alone (Castell *et al.* 1994). It is likely that, at specific stages in the life cycle of fish, higher levels of ARA may be required to cope with periods of environmental stress (Bell & Sargent 2002). The requirement of ARA in fish, however, seems to depend on the fish species and larval stage, and needs to be given with extreme care because it may act in a different way depending on the DHA concentration (Castell *et al.* 1994; Koven *et al.* 2000). Studies on ARA in crustaceans are rather scarce. Glencross and Smith (2001) reported that that ARA is not really essential if linoleic acid (18:2 n-6), the natural precursor to ARA, is present in sufficient amounts in diets for *P. monodon*. These authors suggested that possible effects of ARA may rather lie in the importance of the balance of n-3 to n-6 fatty acids in the diet.

Likewise, the MR1 values of the different n-3 HUFA enrichment treatments were not significantly different in experiment 2. Therefore, it seems that the various total n-3 HUFA levels (28–50%) and DHA/EPA ratios (0.6–4) tested in this experiment could all satisfy the requirements for first metamorphosis.

The effects of dietary HUFA on the second metamorphosis rate were more pronounced. Nutritional effects often only become obvious in later stages when the larvae have used up all reserves built up in earlier stages. In this respect, it has been reported that n-3 HUFA are among the last components to be utilized (Galois 1987; cited in Wouters *et al.* 1997), probably because they play an important role as fatty acid groups of polar lipids in cell membranes (Sargent, Bell, Bell, Henderson & Tocher 1993; Watanabe 1993).

In experiment 1, three groups could be distinguished on the basis of their MR2 rates: high survival through second metamorphosis for the treatments with a high DHA/EPA ratio (30/4 and 30/4/ARA, over 90%), intermediate survival for treatments with a low DHA/EPA ratio (50/0.6 and 30/0.6, approximately 70%) and low survival for treatments with a low total n-3 (or total HUFA) content ('unenrich' control and 0/-, approximately 10%) respectively. Following the same pattern as for larval development rate in the zoeal stages, the DHA and total n-3 content, and the DHA/EPA ratio of the *Artemia* were significantly correlated with the second metamorphosis rate.

In experiment 2, no real negative control (low n-3 HUFA) was included. MR2 values were rather similar in all treatments.

Timing and duration of metamorphosis

The onset of metamorphosis was largely dictated by the larval development rate during the zoeal stages. In experiment 1, the treatments using the emulsions containing a medium total n-3 content (30/0.6, 30/4 and 30/4/ARA) reached first metamorphosis earlier and had a shorter metamorphosis period than the treatments with a high (50/0.6) or a low (control and 0/-) total n-3 HUFA content. However, no difference was observed between high and low DHA/EPA treatments. A similar trend was observed for the second metamorphosis.

In experiment 2, the average first metamorphosis time was the shortest for treatment 30/4; intermediate for control and 30/0.6 and significantly longer for 30/0.6–30/4. The duration of metamorphosis was also clearly prolonged in treatments 30/0.6 and 30/4. The average time to reach the second metamorphosis was the longest for treatment 30/0.6–30/4, the shortest in the 'Culture Selco' control and intermediate for 30/0.6 and 30/4. The survival rate may have affected the duration of the metamorphosis. In this respect, i.e. treatment 30/4 had much higher survival, which probably resulted in a wider variation in metamorphosis time in contrast to i.e. treatment 30/0.6, which had very few survivors, but which went through metamorphosis rather uniformly.

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Overall survival from Z1 to megalopa and first crab

In our study, dietary HUFAs did not always result in an instant response on larval performance. However, due to the accumulation of responses there is usually an inflection point where larval survival or development diverges between different treatments (Mann *et al.* 2001). Using *Penaeus stylirostris* as a test organism, it appeared that the EPA and DHA contents in the zoea diet only showed a major impact on survival and development in later stages, when animals had already been switched to another diet (Léger, Bieber & Sorgeloos 1985). The effects of the quality of nutrition during the early larval stages are often manifested only during the later stages of development, particularly upon metamorphosis to the postlarval stages (megalopa and crabs) (Sulkin 1978; Harvey 1996; Jeffs, Willmott & Wells 1999; Ribeiro & Jones 2000; Davis 2003). Therefore, the overall survival rates to megalopa or crab instar are good means to evaluate the final effect of treatments. The overall survival rates of crab instar are also the primary concern for hatchery managers. Our results showed that larval responses to a nutritional factor are not expressed immediately. However, although effects were perhaps not immediately evident, all larval stages should be fed properly enriched live feed in order to build up sufficient HUFA reserves to obtain high survival to the crab stage. This confirms the results of Takeuchi *et al.* (2000) that *S. paramamosain* larvae require both n-3 HUFA-enriched rotifers and *Artemia* nauplii.

In our study, it has been clearly shown that the overall survival rates to megalopa and crab stage were significantly higher in the treatment with a high DHA/EPA ratio. Similarly, for *S. paramamosain*, a high survival rate and maximum carapace width of the first crab stage were obtained when the larvae were fed DHA-enriched *Artemia* (Takeuchi 2000). The higher M/Z1 and C1/Z1 survival rates in treatment 30/0.6–30/4 compared with those of 30/0.6 prove that the crab larvae can recover their development at later stages when DHA-rich *Artemia* was offered from the Z3 stage onwards. However, survival rates to M and C1 in treatment 30/0.6–30/4 were still lower than those of 30/4 and the 'Culture Selco' control. This proves that an emulsion with a DHA/EPA level lower than 1 should not be used for live food enrichment for early stages.

Conclusions and suggestions

The ICES emulsions influenced the fatty acid profiles of the live food and, in turn, the fatty acid profiles of the crab larvae. No differences in survival in the zoeal stages were found between the different enrichment treatments tested. The 'nutritional impact' of HUFAs on the zoeal survival may have been obscured by other more decisive factors such as the larval quality, microbiota and zoo techniques. The significantly lower metamorphosis success in the low-HUFA treatments proved however, that HUFA-rich emulsions for live food enrichment are needed to attain high survival to the crab stage. It appears that the total n-3 level is not as critical as the DHA level and the DHA/EPA ratio.

The larval development rate was very much affected by the dietary n-3 HUFA level and its DHA/EPA ratio. In both experiments, the LSI values of treatments 30/4 tended to be higher than those of 50/0.6 and 30/0.6 throughout the zoeal stages, indicating that a DHA/EPA ratio of 0.6 in enrichment emulsions is not sufficient to support larval development. Based on the results of experiment 2, a DHA/EPA ratio of approximately 1 may be sufficient for Z1–Z2. Because there has been evidence that DHA/EPA requirements may change during development, the specific requirements for each larval stage should be investigated as a function of the diet type (rotifers, *Artemia*, micro-bound diets).

For the total n-3 HUFA content, no differences were found between live food enrichment products containing 30% or 50% n-3 HUFA. Hence, an inclusion level of 30% therefore seems to be sufficient.

Supplementation of ARA had no effect on survival or development during the zoeal stages, but the first metamorphosis rate was improved by the addition of dietary ARA. Research on the suitable levels of ARA in the enrichment diet for crab larvae needs further investigation.

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