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Effects of salinity and dietary DHA (22:6*n*-3) content on lipid composition and performance of *Penaeus kerathurus* postlarvae

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Abstract A two-way ANOVA experiment was designed to study the effects of salinity and dietary docosahexaenoic acid (DHA; 22:6*n*-3) on lipid composition and performance of postlarvae from the marine shrimp *Penaeus kerathurus* (Forskål, 1775). Shrimp were reared from 1- to 8-d-old postlarvae at 35 and 25‰S with Kelko-enriched *Artemia* sp. [20.0 μg (*n*-3) HUFA mg^{-1} dry weight; 9.1 μg DHA mg^{-1} dry weight] and nonenriched *Artemia* sp. [14.2 μg (*n*-3) HUFA mg^{-1} dry weight; 0.3 μg DHA mg^{-1} dry weight]. Dietary DHA content did not affect either total length or survival but influenced the nutritional status represented by condition indices (triacylglycerol/total polar lipid and triacylglycerol/free cholesterol) of 8-d-old postlarvae at the end of the experiment. Culture salinity affected final total length and condition indices but did not show any effect on survival in the different experimental treatments. The interaction of dietary DHA and culture salinity was not significant for total length and survival but was significant for both condition indices used. *P. kerathurus* 8-d-old postlarvae showed better growth, survival and nutritional condition when reared at 35‰S and when fed on Kelko-enriched *Artemia* sp., but the differences with postlarvae from other treatments were very poorly marked. The results demonstrate that 8-d-old postlarvae may have sufficiently developed osmoregulatory capabilities to resist 25‰S under good conditions, although (*n*-3) HUFA-enriched diets may also enhance osmotic stress resistance, general performance and disease resistance.

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Introduction

The penaeid life cycle includes several different stages found in a variety of habitats. While adults are usually found off-shore at higher salinities and greater depths, larval stages inhabit plankton-rich surface waters with an on-shore migration as they develop. The postlarvae of most *Penaeus* species prefer estuaries, or estuarine-like environments, and may ascend a river where salinity is very low (Dall et al. 1990; Minello and Zimmerman 1991). Early planktonic postlarvae migrate vertically on a diel pattern and, as they enter estuaries, are in the water column during the flood tide. Inshore shallow water habitats of many postlarval and juvenile penaeids are generally either continuously or intermittently low in salinity as are many nursery areas. Osmoregulatory capability during ontogeny has been found to be related to size and age, decreasing from nauplii through metamorphosis to postlarvae followed by an increase in salinity tolerance up to late postlarval stages (Biesiot and Venkataramich 1974; Charmantier et al. 1988; Charmantier-Daures et al. 1988). However, unless there are sudden changes in salinity, the energy cost of osmoregulation over a wide range of salinities is very small in penaeids (Bishop et al. 1980; Liao and Murai 1986). The development of osmoregulation in penaeid postlarvae is also related to the development of the gill morphology which is not complete till late stages, and this is related to diet – better fed postlarvae progressing faster through moults and completing development of more gills.

The pelagic development of the larvae of decapod crustaceans involves a sequence of moult and intermoult periods during which the feeding regime appears to be the determinant of success (Anger and Dawirs 1981; Gore 1985; Ouellet et al. 1992). It has been suggested that food energy is partitioned into survival, moulting and growth, with priority placed on survival (Knowlton 1974). Thus, successful larval development and metamorphosis depend on efficient utilization of energy reserves, especially lipids (Holland and Spencer 1973;

Holland 1978; Sasaki et al. 1986; Whyte et al. 1987; Fraser 1989; Ouellet et al. 1992). Moreover, lipid content, and particularly triacylglycerol (TAG) content, has been used as a measure of the nutritional status and survival potential of decapod larvae (Håkanson 1984; Fraser 1989; Ouellet et al. 1992, 1995; Lovrich and Ouellet 1994; Mourente et al. 1995). The unique life cycle of penaeid larvae, with a long series of planktotrophic stages involving a change in trophic levels, has made nutritional research difficult (Jones et al. 1993), but most research has focused on lipid levels, with increased survival and growth observed when feeding *n*-3 highly unsaturated fatty acid (HUFA)-enriched diets, primarily eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) (Sorgeloos and Léger 1992). These fatty acids are particularly abundant in most species of marine phyto- and zooplankton (Sargent et al. 1989, 1990). Essential fatty acid requirements have been reported for crabs (Levine and Sulkin 1984) and shrimp (Kanazawa et al. 1979a, b, 1985, Read 1981; Rees et al. 1994), and it has been demonstrated that high dietary levels of (*n*-3) HUFA confer resistance to hypo-osmotic stress upon postlarvae (Sorgeloos 1989; Tackaert et al. 1989; Sorgeloos and Léger 1992). Since penaeid larvae have a limited ability for bioconversion of 18:3(*n*-3) to EPA and DHA (Teshima et al. 1992), these pre-formed fatty acids must be added to larval diet.

Penaeus kerathurus is distributed in the eastern Atlantic (from Portugal to Angola) and the Mediterranean Sea (Holthuis 1952). One of the principal aggregations of *P. kerathurus* spawners is located in the Atlantic, close to the mouth of the Guadalquivir River (southwest Spain). In spring, adult mature and mated females migrate from deeper off-shore waters to the shallower spawning-ground waters where the first pelagic larval stage is released into the water column before migration into the estuary of the Guadalquivir River during ontogeny (Rodríguez 1985, 1986, 1987). *P. kerathurus* is one of the less studied penaeid species, as it is difficult to culture due to high mortalities, mainly at early postlarval stages, the so-called "critical phase", which is characterized by transition from pelagic to benthic life (Rodríguez 1975).

The objectives of the present study, based on laboratory observations, were (1) to determine how factors such as salinity and dietary DHA (22:6 *n*-3) affect postlarva condition (growth, survival and nutritional status), (2) to provide by laboratory experience a better understanding of the migration of *Penaeus kerathurus* early postlarvae to estuarine zones, and (3) to improve the culture of this species during the critical, early postlarval stages.

Materials and methods

Culture of *Penaeus kerathurus* larvae

Wild mature and mated females of *Penaeus kerathurus* (Forskål, 1775) were obtained live from a commercial trawler. They were caught in the Gulf of Cádiz (southwest Spain) in the spawning area at the mouth of the Guadalquivir River (Rodríguez 1976). Gravid females were placed in 150-liter rectangular tanks with filtered seawater (salinity 32 ± 1 ‰, temperature 25 ± 1 °C). The treatment to induce spawning consisted of the addition of 1 mg EDTA-Na_2 l^{-1} (to avoid collapse of eggs) and raising the temperature to 27–28 °C. Spawning took place during the night, and the next morning normal and abortive spawns were found. Viable eggs hatched 24 to 36 h later at 25 ± 1 °C with a hatching rate of 60%. Nauplii were concentrated to a point of light and collected by siphon and then reared in a 150-liter cylindroconical tank at a density of 100 individuals l^{-1}, salinity 35 ± 1 ‰ and temperature 26 ± 1 °C. Larvae were reared from the later naupliar stages to Protozoea 2 (PZ_2) using the marine microalga *Skeletonoma costatum* (Greville) Cleve at densities of approximately 60 cells μl^{-1}. From Protozoea 3 (PZ_3) to Postlarva 1 (PL_1), freshly hatched *Artemia* sp. nauplii, originating from Artemia System AF Grade, were added at 5 to 10 individuals ml^{-1}. The survival recorded at Postlarva 1 (PL_1) was 85.6%. At this point, two replicates corresponding to four different experimental treatments: two different culture salinities (25 and 35‰) and two dietary regimes (with and without DHA) were prepared as described in Table 3. Rearing containers were 20-liter clear acrylic tubes fitted with conical bases and lowest-point aeration. Water renewal was 100% d^{-1}, thorough aeration, and a photoperiod of 16 h light:8 h dark was maintained throughout the experiment. Each experimental container was seeded with 1000 1-d-old postlarva (PL-I) at the beginning of the experiment. Samples of 8-d-old postlarvae (PL-V developmental stage) were collected for biometric determinations (total length and individual dry weight) and biochemical analysis.

Culture of phytoplankton and zooplankton

The marine microalgae *Skeletonoma costatum* (Greville) Cleve was grown to 2–3 × 10^6 cells ml^{-1} culture density in semicontinuous batch culture in UV-irradiated, well-sourced seawater filtered to 1 µm, salinity 32 ± 1 g l^{-1} and supplemented with *f*/2 growth medium (Guillard 1975). *Artemia* sp. metanauplii were hatched from Artemia System AF Grade strain cysts (Inve Aquaculture, Belgium) and were enriched with spray-dried cells of *Schizochytrium* sp., manufactured by Kelko (San Diego, California). The enrichment dose consisted of 300 mg of the spray-dried cells per 200 000 metanauplii and per liter during 8 h with strong aeration. Samples of enriched and nonenriched metanauplii were collected for biochemical analysis.

Determination of total length, dry weight, ash, total protein and total carbohydrate contents

Triplicate samples of 20 PL_8 were used for total length determination using a binocular microscope. Triplicate samples of 50 PL_8 washed in distilled water were deposited on a tared glass cover slide and oven dried at 110 °C for 24 h for individual dry weight determination. After cooling in vacuo for at least 1 h, samples were weighed using a Mettler UM3 microbalance. Ash content was measured gravimetrically after total combustion in a furnace at 550 °C. Protein content was measured by the Folin-phenol reagent method, according to Lowry et al. (1951). Total carbohydrate content was determined by a colorimetric method using phenol-sulphuric acid reagent (Dubois et al. 1956).

Total lipid extraction and quantification

Lipids were extracted from preweighed samples by homogenization in chloroform/methanol (2:1, v/v), containing 0.01% (w/v) butylated hydroxytoluene (BHT) as an antioxidant, according to Folch et al. (1957). Solvent was evaporated under a stream of nitrogen and lipid extracts desiccated overnight in vacuo before their mass was determined gravimetrically. Lipid extracts were redissolved in chloroform/methanol (2:1, v/v + BHT) at a known concentration

and stored under an atmosphere of nitrogen at −20 °C until analysis.

Lipid class separation and quantification

Lipid classes were separated by high-performance thin layer chromatography (HPTLC) using a single-dimension, double-development method described previously (Tocher and Harvie 1988). The classes were quantified by charring followed by calibrated densitometry using a Shimadzu CS-9001PC dual-wavelength flying spot scanner (Olsen and Henderson 1989).

Fatty acid analysis

Fatty acid methyl esters from total lipids were prepared by acid-catalyzed transmethylation for 16 h at 50 °C, using nonadecanoic acid (19:0) as internal standard (Christie 1989). The fatty acid methyl esters were analyzed in a Hewlett-Packard 5890 A Series II gas chromatograph equipped with a chemical bonded (PEG) Omegawax 320 fused-silica wall-coated capillary column (30 m × 0.32 mm i.d.) (Supelco Inc., Bellefonte, USA), using hydrogen as carrier gas with a thermal gradient from 185 to 235 °C. Individual fatty acid methyl esters were identified and quantified by reference to authentic standards and to a well-characterized fish oil, using Hewlett-Packard 3365 ChemStation software in a computer linked to the gas chromatograph. All solvents contained 0.01% BHT as an antioxidant.

Available energy

Diet-available energy was calculated by using the energy conversion factors 18.0, 35.2 and 17.2 kJ g^{-1} dry weight, for proteins, lipids and carbohydrates, respectively (Baukema and De Bruin 1979).

Experimental design and statistical analysis

Results are presented as means ± SD (n = 3). The effects on the culture performance variables [total length, survival, and the nutritional indices triacylglycerol (TAG), total polar lipid (TPL) and TAG/free cholesterol (C)] of 8-d-old postlarvae (PL-V developmental stage) due to different culture salinities and different dietary DHA contents were analyzed by Model I two-way ANOVA. Differences among means were analyzed by one-way ANOVA, followed when pertinent by a multiple comparison test (Tukey). Differences were reported as statistically significant when $P < 0.05$ (Zar 1984).

Materials

Potassium bicarbonate, potassium chloride, cupric acetate, BHT and nonadecanoic acid (> 99% pure) were from Sigma Chemical Co. Ltd. TLC (20 × 20 cm × 0.25 mm) and HPTLC (10 × 10 cm × 0.15 mm) plates precoated with silica gel 60 (without fluorescent indicator) and all solvents (analytical grade) were obtained from Merck, Darmstadt (Germany). Glacial acetic acid, sulphuric acid and orthophosphoric acid were purchased from Fluka Chemicals Co. Ltd.

Results

Gross composition, energy content and lipid class composition of enriched and nonenriched *Artemia* sp. metanauplii, as well as the enricher used, are presented in Table 1. Enriched *Artemia* sp. showed a significantly higher proportion of total neutral lipids due to the high TAG content in the enricher. The fatty acid content of

Table 1 Dry weight, gross composition (dry weight percentage), energy level (kJ g^{-1} dry weight) and lipid class composition (total lipid percentage) of the diets used to feed postlarvae. Data are means ± SD (n = 3). SD = 0.0 implies an SD < 0.05. Values within a given row not bearing the same superscript letter are significantly different at $P < 0.05$. If no superscript appears, values are not different (*nd* not determined)

	Kelko	*Artemia* sp. metanauplii	*Artemia* sp. metanauplii + Kelko
Dry weight	96.2 ± 1.3[a]	5.4 ± 0.3[b]	7.2 ± 0.6[b]
Protein	54.9 ± 0.4[a]	67.4 ± 2.2[b]	65.4 ± 2.3[b]
Lipid	27.2 ± 2.1[a]	20.4 ± 1.8[b]	21.9 ± 1.3[b]
Carbohydrate	9.9 ± 0.4[a]	7.5 ± 0.2[b]	7.5 ± 0.1[b]
Ash	8.0 ± 0.5[a]	4.7 ± 0.3[b]	5.2 ± 0.4[b]
Energy from protein	9.9 ± 0.3[a]	12.1 ± 0.7[b]	11.8 ± 0.8[b]
Energy from lipids	9.6 ± 0.6[a]	7.2 ± 0.5[b]	7.7 ± 0.4[b]
Energy from carbohydrate	1.7 ± 0.2[a]	1.3 ± 0.1[b]	1.3 ± 0.2[b]
Total energy	21.2 ± 0.4	20.6 ± 0.8	20.8 ± 0.7
Phosphatidylcholine	9.9 ± 0.7[a]	16.6 ± 1.3[b]	13.1 ± 0.9[c]
Phosphatidylethanolamine	1.0 ± 0.2[a]	15.9 ± 1.1[b]	10.5 ± 0.7[c]
Phosphatidylserine	2.5 ± 0.4	3.3 ± 0.4	2.6 ± 0.3
Phosphatidylinositol	2.4 ± 0.2[a]	6.3 ± 0.4[b]	4.8 ± 0.3[c]
Phosphatidic acid/cardiolipin	nd	3.4 ± 0.2[a]	4.0 ± 0.2[b]
Phosphatidylglycerol	0.8 ± 0.1	nd	nd
Sphingomyelin	nd	0.9 ± 0.1	1.0 ± 0.1
Pigments	nd	4.5 ± 0.3[a]	2.7 ± 0.2[b]
Lyso-phosphatidylcholine	0.6 ± 0.0	0.8 ± 0.1	0.6 ± 0.0
Monogalactosyldiacylglycerol	1.6 ± 0.2	nd	nd
Digalactosyldiacylglycerol	1.0 ± 0.1	nd	nd
Total polar lipids	19.9 ± 1.1[a]	51.7 ± 2.6[b]	39.5 ± 1.7[c]
Cholesterol	7.9 ± 1.3[a]	16.5 ± 1.8[b]	14.3 ± 2.3[b]
Free fatty acid	3.5 ± 0.4[a]	2.4 ± 0.7[a]	5.1 ± 0.6[b]
Triacylglycerol	56.8 ± 2.4[a]	23.5 ± 1.1[b]	37.2 ± 1.8[c]
Sterol ester	11.8 ± 1.0[a]	5.6 ± 0.4[b]	3.9 ± 0.4[c]
Total neutral lipids	80.1 ± 3.4[a]	48.0 ± 2.1[b]	60.5 ± 2.7[c]

the enricher was characterized by the presence of high levels of the saturated 14:0 and 16:0 and the polyunsaturated 22:5(*n*-6) and 22:6(*n*-3) fatty acids (Table 2). In consequence, enriched *Artemia* sp. presented significantly higher contents of these fatty acids.

The experimental conditions, prey density, feeding regimes used to feed postlarvae, as well as the culture performance of the 8-d-old postlarvae are shown in Table 3. Total length and individual dry weight values tended to be greater in postlarvae reared at 35‰S than those reared at 25‰S, independent of the diet used. However, no significant differences were found in survival percentage between the different treatments.

Gross composition and lipid class content of *Penaeus kerathurus* postlarvae at the end of the experiment are presented in Table 4. Dry weight percentage was significantly higher in postlarvae reared at 35‰S. Postlarvae that had been fed with nonenriched *Artemia* sp. at 35‰S showed the highest proportion of total protein and total carbohydrates but the lowest total lipids. Total polar lipid and total neutral lipid contents were significantly higher in those postlarvae reared at the same salinity and that had been fed with enriched *Artemia* sp. Furthermore, total polar lipid and total neutral lipid contents in postlarvae that had been fed with the same diet were significantly higher in those that had been reared at lower salinity. The same differences were observed in the content of either individual polar lipid classes or individual neutral lipid classes in 8-d-old postlarvae from the four different experimental treatments. Postlarvae condition index based on TAG/TPL and TAG/C ratios indicated a better nutritional status in those individuals that had been reared at 35‰S and even better in those postlarvae fed with enriched *Artemia*. sp.

The fatty acid content of 8-d-old *Penaeus kerathurus* postlarvae at the end of the experiment are presented in Table 5. Postlarvae reared at 25‰S and fed with enriched *Artemia* sp. showed the highest values for total saturated, total monoenes and total polyenes. Postlarvae reared at 25‰S and fed with nonenriched *Artemia* sp. and postlarvae reared at 35‰S and fed with enriched *Artemia* sp. presented the second highest values for total

Table 2 Fatty acid content (μg mg^{-1} dry weight) of the experimental diets used to feed postlarvae. Data are means ± SD ($n = 3$). SD = 0.0 implies an SD < 0.05. Values within a given row not bearing the same superscript letter are significantly different at $P < 0.05$. If no superscript appears, values are not different (*HUFA* highly unsaturated fatty acid; *DHA* docosahexaenoic acid; *EPA* eicosapentaenoic acid; *nd* not determined)

	Kelko	*Artemia* sp. metanauplii	*Artemia* sp. metanauplii + Kelko
14:0	32.7 ± 2.5[a]	1.5 ± 0.3[b]	4.4 ± 0.3[c]
15:0	2.3 ± 0.1	2.5 ± 0.2	2.0 ± 0.1
16:0	78.5 ± 5.7[a]	11.2 ± 0.8[b]	18.9 ± 1.3[c]
16:1(*n*-7)	10.9 ± 1.1	8.9 ± 0.5	9.7 ± 0.6
16:2	0.4 ± 0.1	0.5 ± 0.0	0.4 ± 0.1
17:0	0.4 ± 0.0[a]	3.0 ± 0.4[b]	2.3 ± 0.2[c]
16:3	0.8 ± 0.1[a]	7.8 ± 0.5[b]	6.2 ± 0.4[c]
16:4	0.4 ± 0.0	0.7 ± 0.2	0.6 ± 0.1
18:0	3.1 ± 0.4[a]	7.0 ± 0.6[b]	5.8 ± 0.4[c]
18:1(*n*-9)	6.0 ± 0.4[a]	14.3 ± 0.9[b]	10.9 ± 0.8[c]
18:1(*n*-7)	nd	13.1 ± 1.1	10.9 ± 0.9
18:2(*n*-6)	nd	3.2 ± 0.2[a]	2.5 ± 0.3[b]
18:3(*n*-3)	nd	0.9 ± 0.1	0.7 ± 0.1
18:4(*n*-3)	0.4 ± 0.1[a]	1.4 ± 0.3[b]	1.2 ± 0.2[b]
20:0	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
20:1(*n*-9)	0.8 ± 0.1[a]	0.4 ± 0.1[b]	0.3 ± 0.1[b]
20:1(*n*-7)	nd	0.5 ± 0.1	0.4 ± 0.1
20:2(*n*-6)	nd	0.8 ± 0.1[a]	0.4 ± 0.1[b]
20:3(*n*-6)	nd	0.3 ± 0.0	0.4 ± 0.1
20:4(*n*-6)	1.1 ± 0.2[a]	3.9 ± 0.3[b]	3.8 ± 0.3[b]
20:3(*n*-3)	nd	0.7 ± 0.1[a]	0.4 ± 0.0[b]
20:4(*n*-3)	nd	0.4 ± 0.1	0.5 ± 0.1
20:5(*n*-3)	1.7 ± 0.3[a]	10.8 ± 0.7[b]	9.3 ± 0.7[c]
22:0	3.5 ± 0.4[a]	0.6 ± 0.1[b]	0.5 ± 0.0[b]
22:1	nd	0.9 ± 0.1	1.1 ± 0.2
22:5(*n*-6)	24.6 ± 1.8[a]	3.1 ± 0.2[b]	5.8 ± 0.3[c]
22:5(*n*-3)	nd	1.6 ± 0.2[a]	0.4 ± 0.1[b]
22:6(*n*-3)	50.4 ± 2.8[a]	0.3 ± 0.0[b]	9.1 ± 0.7[c]
Total saturated	122.0 ± 8.7[a]	26.2 ± 1.8[b]	34.4 ± 2.5[c]
Total monoenes	17.7 ± 0.9[a]	38.2 ± 2.3[b]	33.6 ± 2.5[b]
Total polyenes	79.8 ± 5.6[a]	34.9 ± 2.2[b]	42.1 ± 2.5[c]
Total (*n*-9)	6.8 ± 0.7[a]	15.1 ± 0.9[b]	11.9 ± 0.6[c]
Total (*n*-7)	10.9 ± 0.8[a]	22.5 ± 1.3[b]	21.1 ± 0.9[b]
Total (*n*-6)	26.1 ± 1.3[a]	9.8 ± 0.7[b]	13.3 ± 0.8[c]
Total (*n*-3)	53.7 ± 2.5[a]	25.1 ± 1.7[b]	28.8 ± 2.1[c]
HUFA (*n*-6)	25.7 ± 1.4[a]	5.4 ± 0.6[b]	10.0 ± 0.6[c]
HUFA (*n*-3)	52.1 ± 2.4[a]	14.2 ± 1.1[b]	20.0 ± 1.2[c]
DHA/EPA	29.6	0.02	0.9

Table 3 *Penaeus kerathurus*. Experimental conditions, prey density, feeding regimes used to feed postlarvae (PL-I–IV) and culture performance of 8-d-old postlarvae (PL-V developmental stage). Data are means ± SD (n = 20 and n = 50). SD = 0.0 implies an SD < 0.05. Values within a given row not bearing the same superscript letter are significantly different at $P < 0.05$. If no superscript appears, values are not different

	Treatment			
	A	B	C	D
Experimental conditions				
Temperature (°C)	26 ± 1	26 ± 1	26 ± 1	26 ± 1
Salinity (‰)	35	35	25	25
Number of postlarvae PL-I per tank at the begining of the experiment	1000	1000	1000	1000
Prey density (postlarva^{-1})				
PL-I	200–250	200–250	200–250	200–250
PL-II	200–300	200–300	200–300	200–300
PL-III	300–400	300–400	300–400	300–400
PL-IV	500	500	500	500
Feeding regime				
Food type	*Artemia* sp. metanauplii	*Artemia* sp. metanauplii	*Artemia* sp. metanauplii	*Artemia* sp. metanauplii
Enrichment	not enriched	Kelko enriched	not enriched	Kelko enriched
Characteristics of 8-d-old postlarvae (PL-V developmental stage) at the end of the experiment				
Total length (μm)	7183.2 ± 137.5^{a}	7117.6 ± 150.3^{ab}	7002.8 ± 164.8^{b}	7072.5 ± 158.7^{ab}
Individual dry weight (μg)	369.4 ± 29.7^{a}	350.2 ± 26.7^{ab}	288.6 ± 21.9^{b}	331.8 ± 25.4^{ab}
Survival (%)	87.5 ± 2.3	87.3 ± 1.7	85.6 ± 4.2	85.2 ± 0.8

Table 4 *Penaeus kerathurus*. Dry weight, gross composition (dry weight percentage), lipid class content (μg postlarva^{-1}) and condition indices (triacylglycerol/total polar lipid and triacylglycerol/cholesterol) of 8-d-old postlarvae (PL-V developmental stage). Data are means ± SD (n = 3). SD = 0.0 implies an SD < 0.05. Values within a given row not bearing the same superscript letter are significantly different at $P < 0.05$. If no superscript appears, values are not different

	Treatment			
	A	B	C	D
Dry weight	16.0 ± 0.8^{a}	14.7 ± 1.2^{a}	12.2 ± 0.7^{b}	11.5 ± 0.7^{b}
Protein	67.6 ± 0.9^{a}	61.9 ± 1.1^{b}	63.6 ± 1.7^{b}	64.1 ± 1.1^{b}
Lipid	13.3 ± 0.4^{a}	21.7 ± 0.4^{b}	21.2 ± 0.3^{b}	22.6 ± 0.2^{c}
Carbohydrate	5.2 ± 0.2^{a}	4.1 ± 0.2^{b}	4.0 ± 0.2^{b}	3.0 ± 0.2^{c}
Ash	13.9 ± 0.6^{a}	12.2 ± 1.6^{ab}	11.2 ± 1.7^{ab}	10.3 ± 1.0^{b}
Phosphatidylcholine	10.5 ± 0.2^{a}	15.4 ± 0.4^{b}	14.8 ± 0.1^{b}	20.5 ± 0.7^{c}
Phosphatidylethanolamine	7.3 ± 0.3^{a}	11.5 ± 0.2^{b}	10.0 ± 0.6^{c}	13.8 ± 0.0^{d}
Phosphatidylserine	2.5 ± 0.1^{a}	2.8 ± 0.0^{b}	2.6 ± 0.0^{a}	3.4 ± 0.0^{c}
Phosphatidylinositol	1.3 ± 0.1^{a}	2.1 ± 0.0^{b}	1.7 ± 0.0^{c}	2.4 ± 0.2^{d}
Phosphatidic acid/cardiolipin	1.2 ± 0.1^{a}	1.9 ± 0.0^{b}	1.6 ± 0.1^{c}	2.1 ± 0.0^{a}
Sphingomyelin	0.3 ± 0.0^{a}	0.4 ± 0.1^{ab}	0.4 ± 0.1^{ab}	0.5 ± 0.0^{b}
Lyso-phosphatidylcholine	0.6 ± 0.1	0.5 ± 0.0	0.5 ± 0.2	0.5 ± 0.0
Pigments	1.9 ± 0.0^{a}	2.7 ± 0.0^{b}	3.1 ± 0.4^{bc}	3.6 ± 0.1^{c}
Total polar lipids	25.5 ± 0.6^{a}	37.2 ± 0.3^{b}	34.8 ± 0.6^{c}	46.9 ± 0.9^{d}
Cholesterol	10.5 ± 0.7^{a}	15.5 ± 0.1^{b}	15.4 ± 0.1^{b}	21.1 ± 0.4^{c}
Free fatty acid	2.4 ± 0.1^{a}	5.3 ± 0.1^{b}	3.9 ± 0.0^{c}	5.9 ± 0.4^{d}
Triacylglycerol	9.8 ± 0.1^{a}	15.8 ± 0.2^{b}	12.1 ± 0.1^{c}	16.8 ± 0.6^{d}
Sterol ester	0.8 ± 0.1^{a}	0.9 ± 0.1^{a}	0.9 ± 0.2^{a}	1.5 ± 0.2^{b}
Total neutral lipids	23.6 ± 0.6^{a}	37.4 ± 0.3^{b}	32.4 ± 0.6^{c}	45.3 ± 0.9^{d}
Triacylglycerol/Total polar lipid	0.38 ± 0.00^{a}	0.42 ± 0.00^{b}	0.34 ± 0.00^{c}	0.36 ± 0.00^{c}
Triacylglycerol/Cholesterol	0.93 ± 0.04^{a}	1.02 ± 0.00^{b}	0.78 ± 0.00^{c}	0.79 ± 0.01^{c}

saturated, monoenes and polyenes, and there were no significant differences between them. Lastly, postlarvae reared at 35‰S and fed with nonenriched *Artemia* sp. showed the lowest values for fatty acid contents. The highest content of 20:5(n-3) was found in postlarvae that had been reared at 25‰S, independent of whether the *Artemia* sp. were enriched or not. In contrast, the content of fatty acids such as 20:4(n-6), 22:5(n-6) and 22:6(n-3) was higher in postlarvae that had been fed on enriched *Artemia* sp. metanauplii, independent of the salinity in which the individuals were reared. However, the contents were significantly higher in those postlarvae reared at 25‰S

Table 6 presents the F-values from two-way ANOVA of the different variables tested in 8-d-old *Penaeus kerathurus* postlarvae at the end of the experiment. Dietary DHA content affected neither total length nor survival but did influence condition indices of 8-d-old

Table 5 *Penaeus kerathurus*. Fatty acid content (μg post-larva^{-1}) of 8-d-old postlarvae (PL-V developmental stage). Data are means ± SD (n = 3). SD = 0.0 implies an SD < 0.05. Values within a given row not bearing the same superscript letter are significantly different at $P < 0.05$. If no superscript appears, values are not different (*HUFA* highly unsaturated fatty acid)

	Treatment			
	A	B	C	D
14:0	0.4 ± 0.1^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.4 ± 0.0^{a}
16:0	4.1 ± 0.3^{a}	6.2 ± 0.1^{b}	5.9 ± 0.2^{b}	7.1 ± 0.3^{c}
16:1(*n*-7)	2.1 ± 0.2^{a}	2.8 ± 0.1^{b}	2.9 ± 0.1^{b}	3.1 ± 0.1^{b}
16:2	0.3 ± 0.1^{a}	0.4 ± 0.0^{ab}	0.4 ± 0.0^{ab}	0.5 ± 0.0^{b}
16:3	2.4 ± 0.2^{a}	3.3 ± 0.1^{b}	3.2 ± 0.1^{b}	3.5 ± 0.1^{b}
16:4	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}
18:0	2.6 ± 0.1^{a}	4.0 ± 0.1^{b}	3.5 ± 0.1^{c}	4.5 ± 0.1^{d}
18:1(*n*-9)	4.6 ± 0.2^{a}	6.5 ± 0.1^{b}	6.2 ± 0.1^{b}	7.1 ± 0.3^{c}
18:1(*n*-7)	3.9 ± 0.2^{a}	5.9 ± 0.1^{b}	5.3 ± 0.1^{c}	6.5 ± 0.2^{d}
18:2(*n*-6)	1.2 ± 0.1^{a}	1.6 ± 0.0^{a}	1.6 ± 0.1^{a}	3.2 ± 0.3^{b}
18:3(*n*-3)	0.4 ± 0.0^{a}	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}	1.8 ± 0.1^{c}
18:4(*n*-3)	0.3 ± 0.0^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	0.7 ± 0.0^{c}
20:4(*n*-6)	1.6 ± 0.2^{a}	2.5 ± 0.0^{bc}	2.2 ± 0.1^{c}	2.8 ± 0.1^{c}
20:5(*n*-3)	4.7 ± 0.2^{a}	6.3 ± 0.3^{b}	6.7 ± 0.2^{c}	7.1 ± 0.3^{c}
22:5(*n*-6)	0.1 ± 0.0^{a}	0.5 ± 0.0^{b}	0.2 ± 0.0^{c}	0.7 ± 0.1^{d}
22:5(*n*-3)	0.1 ± 0.0^{a}	0.1 ± 0.0^{b}	0.2 ± 0.0^{c}	0.1 ± 0.0^{b}
22:6(*n*-3)	0.3 ± 0.0^{a}	1.5 ± 0.1^{b}	0.3 ± 0.1^{a}	1.9 ± 0.1^{c}
Total saturated	9.5 ± 0.8^{a}	13.9 ± 0.4^{b}	13.0 ± 0.5^{b}	15.7 ± 0.5^{c}
Total monoenes	11.1 ± 0.7^{a}	16.0 ± 0.2^{b}	15.2 ± 0.3^{b}	17.6 ± 0.6^{c}
Total polyenes	12.1 ± 0.3^{a}	18.8 ± 0.3^{b}	16.8 ± 0.3^{b}	20.8 ± 0.5^{c}
Total (*n*-9)	4.8 ± 0.2^{a}	6.9 ± 0.1^{b}	6.6 ± 0.2^{b}	7.6 ± 0.3^{c}
Total (*n*-7)	6.2 ± 0.4^{a}	9.0 ± 0.1^{b}	8.5 ± 0.2^{b}	9.9 ± 0.3^{c}
Total (*n*-6)	3.3 ± 0.1^{a}	5.2 ± 0.1^{b}	4.8 ± 0.1^{b}	6.0 ± 0.2^{c}
Total (*n*-3)	8.8 ± 0.2^{a}	13.6 ± 0.3^{b}	12.0 ± 0.2^{b}	14.8 ± 0.3^{c}
HUFA (*n*-6)	1.8 ± 0.1^{a}	3.1 ± 0.0^{b}	2.6 ± 0.1^{b}	3.6 ± 0.1^{c}
HUFA (*n*-3)	5.5 ± 0.2^{a}	8.9 ± 0.4^{b}	7.5 ± 0.1^{b}	9.7 ± 0.2^{c}

postlarvae. On the other hand, culture salinity affected final total length and condition indices but did not show any effect on survival of the postlarvae from the different experimental treatments. The interaction of DHA content and culture salinity was not significant for total length or survival but was significant for both condition indices used (TAG/TPL and TAG/C ratios).

Discussion

The ability of penaeid postlarvae to acquire energy resources and effectively partition the energy obtained between maintenance and growth depends on environmental factors. During the early life stages, shrimp migrate to estuarine nursery areas where they experience processes involving behavioural and physiological responses to changing environmental conditions. Salinity is a variable which strongly influences postlarva metabolism although early postlarval stages seem to be characterized by a marked euryhalinity. Osmoregulatory capability varies during development and adapts penaeids to consecutive biotopes. Penaeid postlarvae have been shown to develop a strong hyper-/hypo-osmotic regulation (Charmantier 1987; Charmantier et al. 1994). Hyper-osmoconforming larvae could rely on isosmotic intracellular regulation as they remain at the surface as planktonic stages, while, the higher density hyper-osmotic postlarval stages would be adapted to seek and settle on the bottom (Foskett 1977; Charmantier et al. 1988). Therefore, the osmoregulatory response of the shrimps varies according to the medium, from hypo- to hyper-regulation, and the energy requirement for osmoregulation is thus quite different depending on the external salinity. Moreover, in penaeid shrimp, osmoregulation and ecology are closely linked, although other factors such as bottom selection, food availability, and presence of predators may interfere with the choice of the biotope (Dall 1981).

Several studies, in fish, have indicated that changes in the composition of the lipids in biological membranes affect the movement of water, ions and nonelectrolytes across the cell membranes (Jain and Wagner 1980; Cullis and Hope 1985), and a predominant role in the regulation of the ion absorption properties of membranes has

Table 6 *Penaeus kerathurus*. *F*-values of two-way ANOVA with replication (n = 3 and n = 20) of the variables total length, survival, TAG/TPL ratio and TAG/C ratio, from 8-d-old postlarvae (PL-V developmental stage) after exposure to different experimental conditions (*DHA* docosahexaenoic acid; *TAG* triacylglycerol; *TPL* total polar lipid; *C* free cholesterol)

Source of variation	Total length	Survival	TAG/TPL	TAG/C
DHA content	ns	ns	56.2***	10.7*
Salinity	10.6**	ns	240.2***	149.3***
DHA content × Salinity	ns	ns	20.2**	8.0*

$^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; *ns* not significant ($P > 0.05$)

also been indicated for essential fatty acids (Nonnotte et al. 1987; Finstad and Thomassen 1991). Hyper-regulation requires active ion uptake primarily attributable to cation- and anion-dependent ATPases (Wheatly and Henry 1987), membrane enzymes which can be (directly or indirectly) regulated by membrane lipid and fatty acyl chain compositions (Bell et al. 1985; Chapelle 1986; Sargent el al. 1987; Stanley-Samuelson 1987; Morohashi et al. 1991). On the other hand, high levels of dietary (*n*-3) HUFA have increased survival, growth and resistance to salinity stress in penaeid postlarval stages (Sorgeloos and Léger 1992; Rees et al. 1994).

Experiments in vivo have demonstrated that the rates of synthesis of phosphatidylethanolamine (PE) and phosphatidylserine (PS) in crustacean gills are linked to osmoregulation from seawater to more dilute media (Chapelle 1986), and it has also been suggested that these phosphoglycerides could act as putative regulators of salt transport by contributing to a stabilization of ion channel proteins in fish (Hansen et al. 1992, 1995). PE, the most highly unsaturated of marine animal phospholipids (Sargent et al. 1990, 1991), exhibits a negative charge at neutral pH, and it has been shown that the activity of Na^+/K^+-ATPase is modulated by negatively charged lipids (Sandermann 1978). In the present study, we observed a higher PE content in those postlarvae that had been transferred to low salinity, independent of the diet, but PS content was not significantly different between postlarvae fed with nonenriched *Artemia* sp. and reared at different salinities. On the other hand, PE and PS contents were significantly higher in postlarvae fed with the enriched and more unsaturated *Artemia* sp. A diet with a more unsaturated fatty acid profile could enhance phosphoglyceride synthesis, since phospholipid metabolism makes more extensive demands on a supply of unsaturated fatty acids than does simple acylglycerol metabolism (Gurr and Harwood 1991), and it was evident that postlarvae reared at lower salinity showed higher contents of PE, PS, phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidic acid/cardiolipin (PA/CL). PI content was significantly higher in *Penaeus kerathurus* postlarvae fed on enriched *Artemia* sp. and in specimens reared in low salinity. PI, an arachidonic acid (AA)-rich phosphoglyceride, is thought to play an important role in salt-secreting tissues of marine organisms (Bell et al. 1983; Sargent et al. 1987). PI may be the source of the AA used as precursor for the PGE_2 synthesis, a prostaglandin that is involved in the inhibition of salt secretion in marine teleost gills (Bell et al. 1983) and the acclimation of the marine bivalve *Modiolus demissus* to hypo-osmotic stress (Freas and Grollman 1980; Stanley-Samuelson 1987). Taking into account in the present experiment that, *P. kerathurus* postlarvae reared at low salinity showed a smaller mean size and weight, and significantly lower dry weight contents and condition indices, it is possible that some energy was diverted to phospholipid and prostaglandin synthesis to compensate osmoadaptation to the detriment of growth and/or nutritional status. Also noteworthy is the higher contents of PA/CL in postlarvae reared at low salinity. CL, or diphosphatidylglycerol, is a phosphoglyceride specific to mitochondria in Crustacea (as in other animals), and the synthesis of CL from PA in this organelle may be linked to the formation of ATP, which is necessary for the active transport of Na^+ mediated by the Na^+/K^+-ATPase when postlarvae are acclimated to dilute media (Chapelle and Zwingelstein 1984).

The nutritional condition of larvae, expressed by the TAG/C ratio is closely related to growth and survival expectations during early development of decapod crustaceans (Fraser 1989; Ouellet et al. 1992, 1995; Lovrich and Ouellet 1994). When exogenously derived energy is insufficient to maintain the basal metabolism of the larvae, endogenous TAG is catabolised (Fraser et al. 1987). However, TAG is sometimes catabolised to minor degrees during growth of crustacean larvae (Sasaki et al. 1986), and the accumulation of TAG by the larvae represents the formation of a readily accessible source of energy that can be used during periods of food shortage (Fraser 1989). In the present study, nutritional indices indicated a significantly better response in postlarvae reared at 35‰S and significantly even better in those postlarvae fed enriched *Artemia* sp.

In the present experiment although survival rates obtained were good in all treatments, high dietary (*n*-3) HUFA content did not present a beneficial effect on survival of the postlarvae, and growth promoting effects were not clear either. Moreover, even though enriched *Artemia* sp. contained significantly higher values of (*n*-3) HUFA (20 μg mg^{-1} dry weight) and about 30 times more DHA than nonenriched metanauplii, no significant effects were observed between different treatments aside from those due to nutritional indices. These results do not support some previous studies with penaeid postlarvae by Léger et al. (1985), Millamena et al. (1988) and Rees et al. (1994) but are more in agreement with those presented by Léger et al. (1987) and Abelin (1991). However, it has also been shown that even if growth performance of postlarvae was not improved by feeding HUFA-enriched *Artemia* sp., it considerably enhanced their ability to withstand stressful environmental conditions such as hypo-osmotic shock (Léger and Sorgeloos 1992; Rees et al. 1994). This better resistance could possibly result from higher incorporation of (*n*-3) HUFA in cell membranes and from the improvement in the total physiological condition of the postlarvae (Rees et al. 1994). While, research has been devoted to the role of dietary (*n*-3) HUFA in penaeid postlarvae, not many studies have dealt with the effect of individual (*n*-3) HUFA, such as EPA and DHA. Although Rees et al. (1994) found that *Penaeus monodon* postlarvae may eventually concentrate DHA in preference to EPA and postlarval *P. japonicus* have a greater ability to bioconvert 18:3(*n*-3) to 20:5(*n*-3) and 22:6(*n*-3) (Teshima et al. 1992), there is little understanding of the biological value and essential fatty acid efficiency between EPA and DHA in penaeid postlarvae. Furthermore, in the present

study, *P. kerathurus* postlarvae showed little ability to bioconvert EPA to DHA, since postlarvae fed with nonenriched *Artemia* sp. (containing high levels of EPA, precursor for DHA synthesis) demonstrated a very low content of DHA in body total lipids.

Although *Penaeus kerathurus* postlarvae showed better growth, survival and nutritional condition when reared at 35‰S and when fed on enriched *Artemia* sp. at 25‰S, the differences between treatments were minimal, suggesting that the presence of high levels of DHA (HUFA over 14.2 μg mg^{-1} dry weight and EPA 10.8 μg mg^{-1} dry weight) is not of vital importance for this species. These values fall within the optimal levels of (*n*-3) HUFA (12 and 22 μg mg^{-1} dry weight) proposed for other penaeid postlarvae (Rees et al. 1994). Besides, 8-d-old *P. kerathurus* postlarvae at PL-V developmental stage have sufficiently developed osmoregulatory capabilities to resist 25‰S in good conditions and, in consequence, these postlarvae can perfectly migrate into estuarine habitats to pass from pelagic to benthic life.

However, in relation to larval culture of this species, we recommend that dilute media should be avoided during early postlarval stages, since no marked improvement in the performance of the culture at low salinity was observed. Furthermore, as has been pointed out previously (Léger and Sorgeloos 1992), (*n*-3) HUFA enriched diets may enhance resistance to osmotic stress and disease, a possibility which deserves further study.

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