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Variations in lipid content and nutritional status during larval development of the marine shrimp *Penaeus kerathurus*

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Abstract

Lipid class and fatty acid contents, survival and nutritional status of *Penaeus kerathurus* larvae, reared on a mixture of the marine microalgae *Tetraselmis chuii* and *Isochrysis galbana* (clone T-ISO), the rotifer *Brachionus plicatilis* and *Artemia* (parthenogenetic strain from Cádiz, S.W. Spain), were studied throughout development. Dry weight and free sterol contents increased and correlated positively ($r=0.91$, $P<0.05$) during development, whereas the ratio of triacylglycerol/free sterol (an indicator of the nutritional status of the larvae) decreased as did survival. Fatty acid contents at different larval stages reflected fatty acid content of foods. The content of docosahexaenoic acid (22:6*n*-3) remained constant during the mysis and first postlarval stage and a low bioconversion rate from its precursor, eicosapentaenoic acid (20:5*n*-3) (very abundant in *Artemia* food) was observed.

Keywords: *Penaeus kerathurus*; Larvae – development; Fats and fatty compounds; Feeding and nutrition — crustaceans

Abbreviations: AA, arachidonic acid (20:4*n*-6); CL, cardiolipin; DHA, docosahexaenoic acid (22:6*n*-3); EPA, eicosapentaenoic acid (20:5*n*-3); FFA, free fatty acid; GLY, glycosylglycerides; HUFA, highly unsaturated fatty acid ($\geq$C 20:3); MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid (more than one double bond); S, free sterol; SE, sterol ester; SM, sphingomyelin; SQDG, sulphoquinovosyldiacylglycerol; TAG, triacylglycerol.

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1. Introduction

The pelagic development of the larvae of decapod crustaceans encompasses 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, molting 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). Lipid content and particularly triacylglycerol (TAG) content has been used as a measure of the nutritional status of food for decapod larvae (Håkanson, 1984; Fraser, 1989; Ouellet et al., 1992). 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). Most nutritional research in penaeid larvae has focused on lipid levels, and particularly highly unsaturated fatty acid (HUFA) levels, with increased survival and growth observed when feeding *n*-3 HUFA-enriched diets (primarily EPA and DHA) (Sorgeloos and Léger, 1992). However, only a few biochemical studies on the larval development of penaeid species have been conducted (Ward et al., 1979; Teshima and Kanazawa, 1982; Cahu et al., 1988). *Penaeus kerathurus* is one of the less studied penaeid species, as it is difficult to culture. The main reason is high mortalities, mainly at the postlarval stages, the so-called "critical phase" which is characterized by transition from pelagic to benthic life (Rodríguez, 1975) and few if any nutritional studies have been devoted to the elucidation of the physiology of digestion, nutrient requirements or metabolism in this species.

The objectives of this study were to determine the variations in lipid class and fatty acid content and nutritional status of *P. kerathurus* larvae, fed on a regime of algae, rotifers and *Artemia*, throughout development. At the same time, the performance of the culture was evaluated by measuring the survival percentages at different larval stages.

2. Materials and methods

Culture of P. kerathurus *larvae*

Wild mature *Penaeus kerathurus* females 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 50-l tanks with filtered seawater (salinity 32 ± 1 ppt, temperature 25 ± 1°C). The treatment to induce spawning consisted of the addition of 1 mg EDTA-Na_2/l (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 24–36 h later at 25 ± 1°C and nauplii were concentrated to a point of light and collected by siphon. Larvae were reared from the later naupliar stages to protozoea 2 (PZ_2) using a mixture of the marine microalgae *Tetraselmis chuii* and *Isochrysis galbana* (clone T-ISO) at densities between 100 and 200 cells μl^{-1}. From protozoea 3 (PZ_3) to mysis 1 (M_1) rotifers, *Brachionus plicatilis*, cultured on *I. galbana* were added at densities between 10 and 20 individuals ml^{-1} plus the mixture

of algae used previously. From mysis 2 (M_2) to postlarvae 1 (PL_1), freshly hatched *Artemia* nauplii, originating from the salt-marshes of Cádiz (Mourente and Rodríguez, 1989), at 5–10 individuals ml^{-1} and also the mixture of algae was added. Rearing containers were 150-l clear acrylic tubes fitted with conical bases and lowest point aeration. Initially, 50 000 nauplii were stocked in 50 l of seawater, and the volume of the culture was increased gradually to 150 l. The final densities of the different larval stages were 1000 nauplii/l, 411 PZ_2/l, 130 M_1/l and 20 PL_1/l. The final volume of the culture (150 l) was reached at the M_1 stage and water renewal was 50%/day. A photoperiod of 16 h light:8 h dark was maintained throughout the experiment. Samples of larvae were collected at nauplii, PZ_2, M_1, and PL_1 for dry weight and ash determinations and biochemical analysis.

Culture of phytoplankton

Tetraselmis chuii and *Isochrysis galbana* (clone T-ISO) were grown separately in UV-irradiated, well source seawater filtered to 1 μm, salinity 32 ± 1 ppt, supplemented with 0.2 ml/l commercial plant fertilizer (Nutrileaf, Agrocross S.A.). Culture densities reached 0.8–1.2×10^6 and 7–9×10^6 cells ml^{-1}, during semicontinuous batch culture, respectively. A mixture of 50% in volume of both cultures was added to larval cultures (N_5–PZ_2). Samples were collected for biochemical analysis by centrifugation, washed, frozen in liquid nitrogen, lyophilized and stored at -80°C till analysis (Mourente et al., 1990).

Culture of zooplankton

Rotifers, *Brachionus plicatilis* S-1 strain (Yúfera, 1982), were cultured on *I. galbana* (clone T-ISO). Cysts of a parthenogenetic *Artemia* strain, collected from the salt-marshes of Cádiz, Spain (Roman and Rodríguez, 1989) were hatched overnight in 1-μm filtered, UV-irradiated, well source marine water (salinity 32 ± 1 ppt). Fresh nauplii were added to larval cultures each day after water exchange. Samples of freshly hatched nauplii were also collected for biochemical analysis.

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

Triplicate samples of 300 nauplii, 100 Z_2, 30 M_1, and 20 PL_1 were washed in distilled water and oven-dried at 110°C for 24 h. 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 concentration was measured by the Coomasie-blue dye method, according to Bradford (1976). Total carbohydrate was determined by a colorimetric method using phenol-sulphuric acid (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 dessicated overnight in vacuo before their mass was determined gravimetrically. Lipid extracts were redissolved in chloroform/methanol (2:1, v/v + BHT) at a concentration of 10 mg/ml 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 (overnight), 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°C 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 a Hewlett-Packard 3394 recording integrator (Tocher and Harvie, 1988). 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 dry weight, for protein, lipid and carbohydrate, respectively (Beukema and De Bruin, 1979).

Statistical analysis

Results are presented as means ± s.d. ($n = 3$). Differences among means were analyzed by one-way analysis of variance (one-way ANOVA), followed when pertinent by a multiple comparison test (Tukey). Differences were reported statistically significant when $P < 0.05$ (Zar, 1984).

Materials

All solvents were analytical grade and supplied by Merck, Darmstadt, Germany. 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) were also obtained from Merck, Darmstadt, Germany. Glacial acetic acid, sulphuric acid and orthophosphoric acid were purchased from Fluka Chemicals Co. Ltd.

3. Results

Gross and lipid class compositions (as dry weight percentages) of the different feeding regimes used to rear *P. kerathurus* larvae are presented in Table 1. The mixture of algae was lower in protein content and higher in carbohydrate and ash. The rotifers had the lowest level of total lipid, whereas the mixture of algae and *Artemia* did not show any significant difference. The *Artemia* was intermediate in carbohydrate and lowest in ash content. Total

Table 1
Gross composition (dry weight percentage), energy level (kJ/g dry weight) and lipid class composition (dry weight percentage) in (A) a mixture of the algae *Tetraselmis chuii* and *Isochrysis galbana* (clone T-ISO), (B) the rotifer *Brachionus plicatilis* cultured with the marine alga *Isochrysis galbana* and (C) nauplii of *Artemia* used to feed different larval stages of *Penaeus kerathurus*

	(A)	(B)	(C)
Dry weight	–	**16.2 ± 0.9**	**16.4 ± 1.1**
Protein	30.3 ± 1.2^a	50.3 ± 1.7^b	55.6 ± 3.5^b
Lipid	19.4 ± 0.8^a	16.1 ± 0.6^b	21.4 ± 2.2^a
Carbohydrate	23.9 ± 0.4^a	9.4 ± 0.6^b	14.2 ± 0.6^c
Ash	42.9 ± 1.6^a	23.9 ± 0.6^b	14.2 ± 0.9^c
Available energy (kJ/g dry weight)			
Energy from protein	5.4 ± 0.2^a	9.0 ± 0.3^b	10.0 ± 0.6^b
Energy from lipid	6.8 ± 0.3^a	5.7 ± 0.2^b	7.5 ± 0.8^a
Energy from carbohydrate	4.1 ± 0.1^a	1.6 ± 0.1^b	2.4 ± 0.1^c
Total energy	16.3 ± 0.4^a	16.3 ± 0.5^a	19.9 ± 1.2^b
Total polar lipids	8.2 ± 0.4^a	5.9 ± 0.5^a	9.5 ± 0.9^b
PC	1.2 ± 0.1^a	1.2 ± 0.1^a	2.9 ± 0.3^b
PE/SQDG	1.2 ± 0.1^a	1.5 ± 0.1^b	2.7 ± 0.3^c
PS	0.1 ± 0.0^a	0.1 ± 0.0^a	0.4 ± 0.0^b
PI	0.1 ± 0.0^a	0.6 ± 0.1^b	0.9 ± 0.1^b
PA/CL	–	0.2 ± 0.0	0.8 ± 0.1
PG	1.3 ± 0.1	–	–
MDGD	3.6 ± 0.1	–	–
GLY	–	0.6 ± 0.0^a	0.3 ± 0.1^b
SM	–	0.1 ± 0.0	0.1 ± 0.0
Pigments	–	0.9 ± 0.3	1.3 ± 0.1
Total neutral lipids	11.1 ± 0.2^a	10.2 ± 0.3^b	11.8 ± 0.9^a
S	–	1.4 ± 0.1^a	3.2 ± 0.2^b
S/Pigments	5.7 ± 0.2	–	–
FFA	1.3 ± 0.0^a	1.9 ± 0.2^b	0.3 ± 0.1^c
TAG	1.2 ± 0.4^a	5.5 ± 0.2^b	5.9 ± 0.7^b
SE	1.0 ± 0.1^a	0.9 ± 0.1^a	2.3 ± 0.2^b

Data are means ± s.d. ($n = 3$). s.d. = 0.0 implies an s.d. of < 0.05. Totals include some minor components ($< 0.1\%$) not shown. Values within a given row bearing different superscript letters are significantly different, $P < 0.05$. Values with no superscript are not different. PC, phosphatidylcholine; PE, phosphatidylethanolamine; SQDG, sulphoquinovosyldiacylglycerol; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; CL, cardiolipin; PG, phosphatidylglycerol; MGDG, monogalactosyldiacylglycerol; GLY, glycosylglycerides; SM, sphingomyelin; S, free sterol; FFA, free fatty acid; TAG, triacylglycerol; SE, sterol ester.

energy content in the mixture of algae and rotifers was identical and both were significantly different ($P < 0.05$) from the total energy content of the *Artemia* diet. However, significant differences between the energy derived from protein, lipid and carbohydrate contents in the three diets were observed. The mixture of algae and *Artemia* were significantly higher in total polar lipid and total neutral lipid than the rotifers. However, the mixture of algae and

Table 2
Total lipid fatty acid content (μg fatty acid/mg dry weight) in (A) a mixture of the algae *Tetraselmis chuii* and *Isochrysis galbana* (clone T-ISO), (B) the rotifer *Brachionus plicatilis* cultured with the marine alga *I. galbana* and (C) nauplii of *Artemia* used to feed different larval stages of *Penaeus kerathurus* during development

Fattty acid	(A)	(B)	(C)
14:0	2.7 ± 0.1^{a}	4.7 ± 0.1^{b}	1.6 ± 0.2^{c}
15:0	3.5 ± 0.3^{a}	4.6 ± 0.1^{b}	2.2 ± 0.1^{c}
16:0	8.3 ± 0.1^{a}	9.2 ± 0.2^{b}	15.2 ± 1.1^{c}
17:0	0.3 ± 0.0^{a}	0.8 ± 0.0^{b}	0.5 ± 0.0^{c}
18:0	0.5 ± 0.0^{a}	2.8 ± 0.1^{b}	6.6 ± 0.5^{c}
20:0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
22:0	tr	0.4 ± 0.1	tr
Total saturated	15.5 ± 0.5^{a}	23.5 ± 0.5^{b}	26.4 ± 1.8^{c}
16:1*n*-7	4.1 ± 0.0^{a}	3.7 ± 0.1^{a}	17.2 ± 1.2^{b}
18:1*n*-9	5.1 ± 0.1^{a}	5.4 ± 0.1^{a}	21.9 ± 2.2^{b}
18:1*n*-7	1.8 ± 0.0^{a}	1.8 ± 0.0^{a}	17.3 ± 0.9^{b}
20:1*n*-9	0.2 ± 0.0^{a}	1.5 ± 0.1^{b}	0.4 ± 0.1^{c}
20:1*n*-7	0.3 ± 0.0^{a}	0.7 ± 0.0^{b}	0.3 ± 0.0^{a}
22:1*n*-11	0.1 ± 0.0	0.3 ± 0.0	nd
22:1*n*-9	nd	0.7 ± 0.3	nd
Total monounsaturated	11.7 ± 0.1^{a}	14.7 ± 0.3^{a}	57.1 ± 4.2^{b}
Total *n*-9	5.3 ± 0.1^{a}	7.9 ± 0.1^{a}	22.2 ± 2.1^{b}
Total *n*-7	6.3 ± 0.0^{a}	5.9 ± 0.2^{a}	34.8 ± 2.1^{b}
16:2	0.9 ± 0.0^{a}	0.7 ± 0.1^{a}	1.4 ± 0.2^{b}
16:3	2.8 ± 0.2^{a}	0.5 ± 0.0^{b}	1.2 ± 0.1^{c}
16:4	4.5 ± 0.0^{a}	1.0 ± 0.0^{b}	1.9 ± 0.2^{c}
18:2*n*-6	7.2 ± 0.1^{a}	11.5 ± 0.1^{b}	5.4 ± 0.4^{c}
18:3*n*-3	6.7 ± 0.2^{a}	6.4 ± 0.1^{a}	4.2 ± 0.2^{b}
18:4*n*-3	3.7 ± 0.1^{a}	3.8 ± 0.1^{a}	1.3 ± 0.1^{b}
20:2*n*-6	0.2 ± 0.0^{a}	0.4 ± 0.0^{b}	0.2 ± 0.1^{a}
20:3*n*-6	tr	0.4 ± 0.0	0.1 ± 0.0
20:3*n*-3	0.2 ± 0.0	0.6 ± 0.0	tr
20:4*n*-6	0.7 ± 0.0^{a}	0.9 ± 0.1^{a}	2.3 ± 0.2^{b}
20:4*n*-3	0.1 ± 0.0^{a}	1.7 ± 0.1^{b}	0.4 ± 0.0^{c}
20:5*n*-3	1.7 ± 0.0^{a}	2.0 ± 0.3^{a}	14.4 ± 1.4^{b}
22:2*n*-6	0.1 ± 0.0	0.2 ± 0.0	nd
22:3*n*-6	0.1 ± 0.0	0.2 ± 0.0	nd
22:3*n*-3	nd	0.1 ± 0.0	nd
22:4*n*-6	nd	0.2 ± 0.0	nd
22:4*n*-3	0.1 ± 0.0	0.3 ± 0.0	nd
22:5*n*-6	0.8 ± 0.1	0.3 ± 0.1	nd
22:5*n*-3	0.1 ± 0.0	0.3 ± 0.0	nd
22:6*n*-3	2.3 ± 0.1	1.9 ± 0.2	nd
Total polyunsaturated	32.4 ± 0.3	33.4 ± 0.5	32.9 ± 2.7
Total *n*-6	10.1 ± 0.2^{a}	14.0 ± 0.2^{b}	9.5 ± 0.8^{a}
Total *n*-3	22.3 ± 0.1^{a}	18.8 ± 0.1^{b}	23.4 ± 1.9^{a}

Data are means $\pm$ s.d. ($n = 3$). s.d. = 0.0 implies an s.d. of < 0.05. Values within a given row bearing different superscript letters are significantly different, $P < 0.05$. Values with no superscript are not different. Totals include some minor components not shown. tr = trace value (< 0.05), nd = not detected.

Table 3
Changes in dry weight (μg/larva), gross composition (dry weight percentage) and lipid class composition (mg/g dry weight) in nauplii, zoea, mysis and postlarvae stages of *Penaeus kerathurus* during development

	Stage of development			
	Nauplius	Zoea	Mysis	Postlarvae
Dry weight (μg/larva)	1.2±0.1[a]	12.5±1.1[b]	28.1±1.5[c]	171.0±6.5[d]
Protein	55.6±0.6[a]	58.3±0.8[a]	54.7±2.4[a]	40.9±0.8[b]
Lipid	28.4±2.7[a]	6.6±0.4[b]	10.8±0.5[c]	2.6±0.1[d]
Carbohydrate	7.2±0.2[a]	8.3±0.4[a]	3.6±0.4[b]	3.1±0.2[b]
Ash	8.7±0.2[a]	26.5±1.7[b]	30.6±1.3[b]	53.3±1.8[c]
Total polar lipid	123.5±1.8[a]	33.5±0.7[b]	52.0±3.1[c]	11.4±1.0[d]
Phosphatidylcholine	57.6±4.6[a]	13.4±0.5[b]	22.4±1.3[c]	4.1±0.4[d]
Phosphatidylethanolamine	37.9±0.7[a]	9.8±0.1[b]	15.5±1.1[c]	3.6±0.3[d]
Phosphatidylserine	8.5±0.5[a]	3.9±0.1[b]	5.4±0.4[c]	1.3±0.1[d]
Phosphatidylinositol	8.1±0.2[a]	1.8±0.1[b]	3.2±0.1[c]	0.7±0.0[d]
Phosphatidic acid/cardiolipin	9.0±1.7[a]	3.8±0.0[b]	3.9±0.3[b]	1.2±0.0[c]
Sphingomyelin	2.1±0.1[a]	0.6±0.1[b]	1.4±0.0[c]	0.5±0.1[d]
Pigments	18.9±3.5[a]	6.6±0.0[b]	10.8±0.3[c]	2.4±0.1[d]
Total neutral lipid	137.5±1.8[a]	30.4±0.7[b]	53.1±3.1[c]	14.0±1.0[d]
Cholesterol	39.5±0.9[a]	11.9±0.3[b]	20.9±0.3[c]	5.2±0.3[d]
Free fatty acid	10.9±4.1[a]	1.4±0.2[b]	3.2±0.5[c]	1.3±0.5[b]
Triacylglycerol	47.9±2.6[a]	7.2±0.0[b]	6.9±0.5[b]	0.7±0.1[c]
Steryl ester	39.6±3.3[a]	9.8±0.1[b]	22.1±2.9[c]	6.7±0.7[d]
Triacylglycerol/Free sterol	1.2±0.0[a]	0.6±0.0[b]	0.3±0.0[c]	0.1±0.0[d]
Survival (%)	100	74	46.5	10.8

Data are means±s.d. ($n=3$). s.d.=0.0 implies an s.d. of <0.05. tr=trace amount <0.05%. Values within a given row bearing different superscript letters are significantly different at $P<0.05$. If no superscript appears, values are not different.

the rotifers were lower in major polar lipid classes (PC and PE) than the *Artemia* diet. In contrast, only the mixture of algae was significantly lower in TAG and SE contents.

Total lipid fatty acid contents from the different foods are presented in Table 2. The *Artemia* diet was quantitatively higher in fatty acid content than the mixture of algae or the rotifer diet. This was due to significantly higher amounts of both saturated and monounsaturated fatty acids (primarily 16:0, 16:1*n*-7, 18:1*n*-9 and 18:1*n*-7). No significant difference was found among the total PUFA contents of the three diets. However, some differences were observed among individual PUFAs. Thus, while *Artemia* was nearly 3-fold richer in 20:4*n*-6 and 7-fold richer in 20:5*n*-3 than the other two diets, 22:6*n*-3 was absent.

Changes in gross composition and lipid class contents throughout larval development are shown in Table 3. Dry weight increased by 10-fold from nauplius to zoea, mainly due to increases in protein, carbohydrate and ash contents. From zoea to mysis, dry weight

Table 4
Variations in total fatty acid content (pg fatty acid/μg dry weight) at different stages of *Penaeus kerathurus* larval development

Fatty acid	Larval stages			
	Nauplii	Zoea	Mysis	Postlarvae
14:0	3.9 ± 0.0^{a}	0.6 ± 0.2^{b}	1.5 ± 0.3^{c}	0.2 ± 0.0^{d}
15:0	1.7 ± 0.2^{a}	1.2 ± 0.2^{a}	1.2 ± 0.2^{a}	0.6 ± 0.1^{b}
16:0	30.1 ± 1.4^{a}	8.1 ± 0.4^{b}	11.7 ± 0.7^{c}	3.1 ± 0.2^{d}
17:0	1.1 ± 0.0^{a}	0.2 ± 0.1^{b}	0.3 ± 0.0^{c}	0.1 ± 0.0^{b}
18:0	13.1 ± 0.7^{a}	3.2 ± 0.2^{b}	6.7 ± 0.5^{c}	2.1 ± 0.1^{d}
20:0	0.9 ± 0.1^{a}	0.2 ± 0.0^{b}	0.4 ± 0.0^{c}	0.1 ± 0.0^{b}
Total saturated	51.8 ± 2.4^{a}	13.7 ± 0.9^{b}	22.0 ± 0.9^{c}	6.4 ± 0.2^{d}
16:1*n*-7	10.6 ± 0.5^{a}	0.8 ± 0.1^{b}	3.1 ± 0.2^{c}	0.8 ± 0.1^{b}
18:1*n*-9	19.0 ± 0.8^{a}	4.2 ± 0.2^{b}	4.4 ± 0.4^{b}	2.1 ± 0.1^{c}
18:1*n*-7	7.9 ± 0.4^{a}	1.7 ± 0.1^{b}	4.1 ± 0.2^{c}	1.9 ± 0.2^{b}
20:1*n*-9	1.6 ± 0.1^{a}	0.5 ± 0.0^{b}	0.9 ± 0.1^{c}	0.2 ± 0.0^{d}
20:1*n*-7	1.5 ± 0.1^{a}	0.1 ± 0.0^{b}	0.3 ± 0.0^{c}	0.1 ± 0.0^{b}
22:1*n*-11	0.3 ± 0.0	0.1 ± 0.0	tr	tr
22:1*n*-9	0.2 ± 0.0	tr	0.2 ± 0.0	tr
Total monoenes	41.3 ± 1.7^{a}	7.5 ± 0.5^{b}	13.2 ± 0.9^{c}	5.1 ± 0.2^{d}
16:2	1.6 ± 0.1^{a}	0.3 ± 0.1^{bc}	0.4 ± 0.1^{b}	0.2 ± 0.0^{c}
16:3	1.3 ± 0.2^{a}	0.4 ± 0.1^{bc}	0.6 ± 0.1^{b}	0.3 ± 0.0^{c}
16:4	3.2 ± 0.2^{a}	0.9 ± 0.0^{b}	2.5 ± 0.1^{c}	0.7 ± 0.0^{b}
18:2*n*-6	1.9 ± 0.1^{a}	3.4 ± 0.1^{b}	4.0 ± 0.2^{c}	0.6 ± 0.0^{d}
18:3*n*-3	0.7 ± 0.1^{a}	3.4 ± 0.1^{b}	1.2 ± 0.1^{c}	0.3 ± 0.0^{d}
18:4*n*-3	0.7 ± 0.1^{a}	0.9 ± 0.1^{a}	0.4 ± 0.0^{b}	0.2 ± 0.0^{c}
20:2*n*-6	1.1 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	0.1 ± 0.0
20:3*n*-6	0.3 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	tr
20:3*n*-3	0.2 ± 0.0^{a}	0.6 ± 0.0^{b}	0.4 ± 0.0^{b}	0.1 ± 0.0^{a}
20:4*n*-6	9.2 ± 0.5^{a}	1.3 ± 0.0^{b}	4.5 ± 0.3^{c}	1.2 ± 0.1^{b}
20:4*n*-3	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	1.8 ± 0.1^{b}	0.1 ± 0.0^{a}
20:5*n*-3	22.5 ± 1.2^{a}	4.1 ± 0.1^{b}	12.4 ± 0.9^{c}	4.3 ± 0.2^{b}
22:5*n*-6	0.7 ± 0.0^{a}	0.5 ± 0.1^{a}	0.7 ± 0.1^{a}	0.2 ± 0.0^{b}
22:5*n*-3	1.8 ± 0.1^{a}	0.1 ± 0.0^{b}	2.0 ± 0.1^{a}	0.2 ± 0.0^{b}
22:6*n*-3	12.1 ± 1.9^{a}	4.8 ± 0.1^{b}	6.4 ± 0.5^{c}	1.1 ± 0.1^{d}
Total polyenes	60.7 ± 0.7^{a}	21.8 ± 0.9^{b}	39.2 ± 2.7^{c}	9.8 ± 0.3^{d}
HUFA *n*-6	12.2 ± 0.6^{a}	2.1 ± 0.2^{b}	6.0 ± 0.5^{c}	1.5 ± 0.1^{b}
HUFA *n*-3	37.4 ± 0.7^{a}	9.8 ± 0.3^{b}	23.1 ± 1.6^{c}	5.8 ± 0.2^{d}

Data are means ± s.d. ($n=3$). s.d. = 0.0 implies an s.d. of < 0.05. tr, trace < 0.05. HUFA: highly unsaturated fatty acids > 20:3. Totals include some minor components not shown. Values within a given row bearing different superscript letters are significantly different at $P < 0.05$. If no superscript appears, values are not different.

increased by 2-fold and lipid increased at a greater rate than protein or ash. From mysis to postlarvae, a 6-fold increase occurred due to increments in protein, carbohydrate and ash

content and lipid to a lesser extent. The proportions of total polar lipids, total neutral lipids and pigments did not show significant changes during larval development, but significant quantitative changes were observed. In absolute terms (μg of lipid/larva), total polar lipids increased by 2.8 times from nauplius to zoea, with PS and PA/CL showing the highest increases (by 4.7 and 4.3 times, respectively). However, when the data were presented per unit of dry weight (ng/μg dry weight), a significant decrease of 3.7-fold was observed in total polar lipids (with decreases for PC and PE of 4.3 and 3.8, respectively) due to an increase in ash content (by 3-fold) during this period. From zoea to mysis, total polar lipids increased by 3.5 times with major increases corresponding to PC (by 3.8-fold) and SM (by 5.5-fold). In contrast, on a dry weight basis, the increase in total polar lipids was only 1.5-fold, with increases of 1.7 and 2.3 for PC and SM, respectively. These increases were due to the significant increase in total lipid during this period. From mysis to postlarvae, total polar lipids increased by 1.3-fold (the lowest increase in absolute terms), having similar increases in all individual classes. Total neutral lipids increased by 2.3-fold from mysis to postlarvae. On a dry weight basis, total polar lipids and total neutral lipids decreased by 4.6-fold and 3.8-fold, respectively, with major decreases in PC, PI and TAG. These decreases were mainly due to significant decreases in total protein and total lipid and the significant increase in ash content from mysis to postlarvae. Free sterol content increased (in absolute terms) by 3.1, 3.9 and 1.6-fold from nauplius to zoea, from zoea to mysis, and from mysis to postlarvae, respectively, showing a positive correlation ($r=0.91$, $P<0.05$) with dry weight increase during development. TAG content increased by 1.6-fold and 2.2-fold from nauplius to zoea and from zoea to mysis, respectively, but decreased by 31.6% from mysis to postlarvae. The SE fraction represented a high percentage among individual lipid classes (from 14.1 to 24.6%) and showed increases of 2.6, 5.1 and 1.8-fold from nauplius to postlarvae, respectively. The TAG/S ratio showed a downward trend from 1.2 to 0.1 during larval development. Survival was calculated considering a 100% survival at naupliar stage at the first sampling point. In consequence, the viability of the culture decreased to 74% at zoea, 46.5% at mysis and 10.8% at postlarvae stages, respectively (Table 3).

Variations in fatty acid content at different stages of larval development are presented in Table 4. Total saturated, total monounsaturated and polyunsaturated fatty acids contents increased throughout larval development when expressed in absolute terms (pg fatty acid/larva), but when results were related to dry weight (pg fatty acid/μg dry weight) significant decreases were shown from nauplii to zoea (with maximun decreases for total monoenes and minimum decreases for total polyenes), from zoea to mysis significant increases were observed in all fatty acids and from mysis to postlarvae significant decreases were observed (maximun decrease for total polyenes and minimum decrease for total monoenes). Major individual fatty acids showed the trends mentioned above. However, 18:1*n*-9, a major monoene, did not increase from zoea to mysis stage. Moreover, comparing the changes in major HUFAs during development, DHA was retained to a larger extent than AA and EPA from the nauplii to the zoea stage, showed a lower increase than AA and EPA from zoea to mysis and had the greatest decrease (by 5.8-fold) from mysis to postlarvae.

4. Discussion

The present study was designed to examine the variations in lipid content and nutritional status of *P. kerathurus* larvae reared with live foods throughout development. Many nutri-

tional studies have shown the important role of lipids in the larval development of different penaeid species (Ward et al., 1979; Teshima and Kanazawa, 1982; Teshima et al., 1983, 1986; Kanazawa et al., 1985; Cahu et al., 1988; Sorgeloos and Léger, 1992). While this is routine for juvenile and adult shrimp (Akiyama et al., 1992), there are very few examples of analysis of penaeid larvae in culture and even fewer for wild larvae (Jones et al., 1993). In penaeids, naupliar stages depend largely upon energy reserves, primarily TAG, which is catabolized by the prefeeding larvae until the energetic demands of growth and metabolism are met from exogenous sources. TAG content correlates with the physiological condition of a larva (Håkanson, 1984; Fraser, 1989; Ouellet et al., 1992) and when exogenously derived energy exceeds the metabolic demand of a larva, the excess energy is accumulated as TAG. The ratio of TAG/free sterol has been proposed as a good indicator of the nutritional status for fish, bivalve and crustacean larvae (Fraser, 1989). In our study, *P. kerathurus* larvae showed a downward trend in the TAG/S ratio throughout development. The decrease observed in this index was due to an absolute decrease in the TAG fraction from nauplii to postlarvae. Since TAG contents in the rotifers and *Artemia* nauplii were not significantly different and the energetic content of *Artemia* was significantly higher, the depletion of TAG throughout development, and particularly from mysis to postlarvae, indicates a priority of development and growth over the accumulation of reserves. Thus, the simplest explanation is that insufficient energy, due to low TAG levels, results in larval death at moult during the critical postlarval stages of development (Ouellet et al., 1992). According to Table 3, total lipid and TAG contents decreased from mysis to postlarvae, but so does protein. If protein assimilation is limited during this period, as has been shown for other penaeid species (Jones et al., 1993), mainly due to an impairment in protease activities, then lipid and particularly TAG reserves must be used as the energy supply. In the present case, the comparison of different food algae versus rotifers or *Artemia*, postlarvae could derive more energy from protein than from other nutrients, whereas for the zoea stage, the main available energy source is represented by lipids (Table 1). A sort of sub-starving condition for the mysis and postlarvae stages could be considered, and a state like that could lead to such final biochemical composition. Starved mysis-stage larvae could become more susceptible to mortality and the rate of metamorphosis could be reduced. The low survival rate at the postlarvae stage could be explained by the starving conditions for the larvae. In consequence, the high mortalities observed may be due to higher energy demands, and prey with high nutritional value could be used to improve the general performance of the culture.

Rapid tissue growth may necessitate a source of free sterols for new membrane formation which could be provided at the expense of the steryl ester pool. It is noteworthy that a specific accumulation of sterol esters occurred throughout larval development, reaching 24.6% of total lipids at the postlarvae stage. This is due to the high levels of this particular fraction contained in *Artemia*, which probably exceed the sterol requirement of the larvae.

Fatty acid content at different larval stages reflects to a great extent the fatty acid content of the foods. It is remarkable that DHA, which is absent in *Artemia*, is specifically retained in postlarvae. To what extent DHA can be derived from its precursor EPA by the desaturation/elongation pathway, or is simply specifically retained in phospholipids of the larvae, cannot be deduced from these data. In either case, the presence of DHA in lipids of postlarvae, even when fed on a diet lacking this particular fatty acid, indicates its essentiality for *P. kerathurus* larvae. It is probable that diets containing higher levels of *n*-3 HUFA will

improve growth and survival of this species as has been demonstrated for other penaeid larvae (Sorgeloos and Léger, 1992).

Radiotracer studies have shown that *Penaeus japonicus* can desaturate/elongate 18:3*n*-3 to 20:5*n*-3 and 22:6*n*-3 (Kanazawa and Teshima, 1977; Jones et al., 1979; Kanazawa et al., 1979; Teshima et al., 1992). However, the conversion rates are too low to meet larval requirements. In any case, it has also been shown that *P. japonicus* larvae (especially postlarvae) have a greater ability to bioconvert 18:3*n*-3 to *n*-3 HUFA, such as EPA and DHA, than the juveniles, which suggests a variation in fatty acid metabolism during metamorphosis (Teshima et al., 1992).

It would be interesting to investigate the role of EPA and DHA as essential fatty acids in *P. kerathurus*, as well as the efficiencies of bioconversion of *n*-3 PUFA to *n*-3 HUFA. Inclusion of *n*-3 HUFA at 1–2% level (on a dry diet, weight basis) has been indicated for successful culture of penaeid larvae (Rodríguez et al., 1994). However, these are minimum values, and the presence of higher levels of *n*-3 HUFA and higher polar and neutral lipid fractions (particularly TAG) could enhance the nutritional value and energy content of diets used to rear *P. kerathurus* by improving its nutritional status and general performance in culture (growth and survival), mainly at postlarval stages, the most critical period in the culture of this species.

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