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Variation in the lipid content of wild-caught females of the marine shrimp *Penaeus kerathurus* during sexual maturation

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Abstract. Changes in total lipids, lipid classes and their fatty acid contents were studied in the ovaries and midgut glands of *Penaeus kerathurus* Forskäl females during sexual maturation. The shrimp were captured in the Gulf of Cádiz (southwest Spain) in 1990. The lipid content and fatty acids, in relative terms, increased during ovarian development. The greatest changes occurred between Maturation Stages III and IV. Ovarian lipids were dominated by polar classes, whereas in the midgut gland the major classes were triacylglycerols and sterol esters. The amounts of major fatty acids in ovaries (16:0, 16:1n-7, 18:1n-9, 18:1n-7, 20:5n-3 and 22:6n-3) increased with increasing maturity, but declined slightly between Stages III and IV. The total polar lipid content of the midgut was 5.7% (by dry weight) and its fatty acid composition remained constant during the whole study period. Total lipid content of the midgut gland showed an upward trend during sexual maturation, except between Stages II and III, when a slight decrease was observed. Predominant fatty acids in the midgut gland (16:1n-7, 20:5n-3 and 22:6n-3) displayed a noteworthy decline between Stages II and III, corresponding with the marked increase in total lipid fatty acid content in the ovaries during the same period.

Introduction

The ovaries and eggs of shrimp tend to be relatively rich in lipids compared to the adults. Since juvenile shrimps may have a limited capacity for *de novo* phospholipid synthesis (Teshima et al. 1986) and an inability to synthesise cholesterol (Teshima 1982), the quality of the dietary lipid source may be of great importance in maturation. Several authors have suggested that successful maturation is dependent on diet (Brown et al. 1979, Lawrence et al. 1980, Cahu and Quazuguel 1989). These factors imply that lipids are nutrients important for the maturation of shrimp. Many works have already noted that lipid deposition occurs in the ovaries during natural sexual maturation of various shrimp species (Gehring 1974, Teshima and Kanazawa 1983, Galois 1984, Jeckel et al. 1989, Teshima et al. 1989) and during induced maturation (Teshima et al. 1988a, b, Bray et al. 1990), but there is little information on the quantitative changes that occur in the different lipid classes and their fatty acids during this period. Even less information is available on changes in lipids during ovarian maturation in *Penaeus kerathurus*, an important commercial species from the Atlantic and Mediterranean. Since lipids play an important role as sources of energy and as cell constituents in the processes of spawning, embryogenesis, hatching and early development of crustacean larvae (Holland 1978, Chang and O'Connor 1983), the present study aimed at explaining the variation in the lipid contents of the ovary and midgut gland during sexual maturation in wild *P. kerathurus* females.

Materials and methods

Wild adult female *Penaeus kerathurus* (Forskäl) were obtained from a commercial trawler. They had been caught in the Gulf of Cádiz (south-west Spain), near the mouth of the Guadalquivir river, during the spring and early summer of 1990. Females were transported alive to the laboratory and classified into four different development stages, according to ovary size and colour (Aubson and Patlan 1974, Rodriguez 1977, 1985). Three females were selected for every stage, wet weight and total length were recorded, and the ovaries and midgut glands were then dissected-out. Ovary wet weight, midgut gland wet weight, gonadosomatic index (GI = ovary wet et × 100/total wet wt) and hepatosomatic index (HI = midgut gland wet wt × 100/total wet wt) were calculated. Biometric data are presented in Table 1. Samples were immediately frozen in liquid nitrogen, freeze-dried overnight, ground, and the powder stored at -80 °C until analysis.

Total lipid extraction and quantification

Total lipids were extracted according to the procedure of Folch et al. (1957) and their mass was determined gravimetrically.

Separation of lipid classes

Lipid classes were separated by thin-layer chromatography (TLC), carried out in unlined tanks on 20 × 20 cm glass plates coated with kieselgel G. The separation of polar lipid (PL) and neutral lipid (NL) classes from total lipids was undertaken using separate TLC plates. PL were separated by one-dimensional TLC, according to a modification of the method of Kaulen (1972); plates were pre-developed in ethyl acetate, dried, and then developed in chloroform-methanol-acetic acid-water (50:25:8:1, v/v/v/v) after spotting with lipid samples. NL were separated into lipid classes by one-dimension TLC using hexane-diethyl ether-acetic acid (80:20:2, v/v/v). Lipid classes were visualized with UV light after being sprayed with rhodamine 6G, 1% in methanol (w/v). Individual standards and standard mixtures were spotted alongside lipid samples to compare R_f values.

Fatty acid analysis

Methyl esters were prepared using boron trifluoride-methanol, 14% (Morrison and Smith 1964). Nonadecanoic acid (19:0) was added to the lipid sample before transmethylation, as an internal standard. The fatty acid methyl esters were analyzed in a Hewlett-Packard 5890 gas chromatograph equipped with flame ionization detector and using a wall-coated open tubular (WCOT) capillary column (30 m × 0.53 mm i.d.) coated with 0.25 μm-thick Supelcowax-10 (polydiethylenglycol, PEG) stationary phase (Supelco Inc., Bellefonte, USA). The temperature of the injector port and the detector was 250 °C. Hydrogen was used as the carrier gas (8 ml/min rate flux) and the oven temperature program consisted of a monophasic thermal gradient from 180 to 220 °C at a rate of 3 C°/min. Individual methyl esters were identified by comparison with the relative retention times of known commercial standards (Supelco, Inc., Bellefonte, USA). Peak integration was carried out with a Hewlett-Packard 3390 A recording integrator. Results are the mean of three different samples for every organ and stage.

Quantification of free cholesterol and polar and neutral lipid classes

Free cholesterol (C) was determined by a colorimetric assay according to Zak et al. (1954), as modified by Henly (1957). Quantitative determination of PL and NL classes was performed by a gas-chromatographic procedure according to Christie et al. (1970), Morrison et al. (1980) and Christie (1982). Lipid class bands, after separation and identification, were scraped off and directly transmethylated after the addition of a known amount of internal standard (19:0). The fatty acid methyl esters of each of the lipid classes were subjected to gas chromatographic (GC) analysis and the amount of every sample was determined by relating the total area of the fatty acid peaks to the peak for the standard. The non-fatty acid material weight in each of the lipid classes was computed by multiplying each result by a factor calculated by dividing the molecular weight of the nonadecanoic acid derivative of the lipid class by the molecular weight of the methyl nonadecanoate. The calculated factors for the individual lipid classes were: polar lipids (PL) = 1.61; monoacylglycerol (MAG) = 1.29; diacylglycerol (DAG) = 1.14; free fatty acids (FFA) = 0.98; triacylglycerol (TAG) = 1.08; sterol esters (SE) = 2.26; lysophosphatidylcholine (LPC) = 1.76; sphingomyelin (SM) = 2.44; phosphatidylcholine (PC) = 1.31; phosphatidylinositol (PI) = 1.42; phosphatidylserine (PS) = 1.31; phosphatidylethanolamine (PE) = 1.24; phosphatidic acid/cardiolipin (PA/CL) = 1.54.

Materials

All solvents were Analar grade and supplied by Merck, Darmstadt, Germany. Butylated hydroxytoluene (BHT) and ferric trichloride were supplied by the Sigma Chemical Co. Ltd. Lipid standards and the GC capillary column were from Supelco Inc., Bellefonte, USA. The TLC plates (20 × 20 cm, 0.25 mm thick), precoated on Silicagel 60 G, were also from Merck, Darmstadt.

Results

Total lipid and lipid-class contents in ovary and midgut gland

Ovarian wet weight of *Penaeus kerathurus* increased by 42.8% from Stages I to II, by 50% between Stages II and III and by 54.8% from Stages III to IV. In contrast, midgut gland weight remained essentially constant during sexual maturation. As the gonadosomatic index increased from Stage I to IV (Table 1), the total lipid, total PL, total NL and the different lipid classes contents in the ovary also increased (Table 2). The total lipid content of the ovaries rose from 11.7 to 24.6% of the dry weight between Maturation Stages I and IV. At Stage I, the total PL content of the ovaries was twice that of total NL, but during maturation total NL was deposited more rapidly than total PL. By Stage IV, the PL and NL content of the ovaries were almost equal, ~12% of the dry weight.

The predominant lipid classes of ovarian total lipids were PC (3.5 to 6.5% of dry wt), PE (0.3 to 1.8%), TAG (1.4 to 5.9%) and SE (0.6 to 2.1%). PC concentrations attained maximum values at Stage III (increased by 46.1%), and then declined slightly to Stage IV (decreased by 4.6%). PE and TAG increased from Stage I to Stage IV by 83.3 and 76.3% of their initial contents, respectively. The cholesterol (C) fraction increased by a factor of ~4 from Stages II to IV (Table 2).

In the midgut gland, total lipids accumulated between Stages I and II (mainly due to deposition of NL), but subsequently decreased substantially between Stages II and III. From this stage onwards, total lipid, total PL and total NL levels remained constant. Total PL content did not vary between developmental stages during maturation, but total NL increased between Stages I and II,

Table 1. *Penaeus kerathurus*. Biometric data for females at different stages (I to IV) of sexual maturation. Data are means ± SD of three different samples for each stage. Total wt, ovary wt and midgut gland wt are in grams, total lengths in millimetres. GI: gonadosomatic index; HI: hepatosomatic index

Parameter	Stages			
	I	II	III	IV
Total wt	25.0±5.2	28.5±8.2	26.5±5.4	26.9±4.0
Total length	148.6±10.9	153.6±11.9	150.6±9.8	151.8±9.3
Ovary wt	0.4±0.1	0.7±0.1	1.4±0.4	3.1±0.5
Midgut gland wt	0.8±0.1	0.9±0.2	1.0±0.1	1.0±0.1
GI	1.8±0.1	2.6±0.3	5.2±0.6	11.8±0.3
HI	3.6±0.3	3.2±0.2	4.0±0.3	3.8±0.2

Table 2. *Penaeus kerathurus*. Total lipid (TL), total polar lipid (TPL), total neutral lipid (TNL), polar lipid and neutral lipid contents in ovaries of females at different stages of sexual maturation. Data are means ± SD of three different samples for each stage, expressed as percentage dry wt. LPC: lysophosphatidylcholine; SM: sphingomyelin; PC: phosphatidylcholine; PI: phosphatidylinositol; PS: phosphatidylserine; PE: phosphatidylethanolamine; PA: phosphatidic acid; CL: cardiolipin; MAG: monoacylglycerol; DAG: diacylglycerol; C: cholesterol; FFA: free fatty acid; TAG: triacylglycerol; SE: sterol esters

Lipid classes	Stages			
	I	II	III	IV
Total				
TL	11.7±0.1	12.3±0.2	17.5±0.3	24.6±0.4
TPL	7.9±0.1	8.5±0.1	11.1±0.2	12.5±0.0
TNL	3.8±0.1	3.7±0.1	6.6±0.2	12.1±0.0
Polar				
LPC	0.6±0.0	0.4±0.1	0.5±0.0	0.8±0.0
SM	0.8±0.0	1.4±0.5	0.8±0.0	1.2±0.1
PC	3.5±0.1	3.7±0.1	6.5±0.2	6.2±0.0
PI	0.8±0.1	0.5±0.1	0.3±0.0	0.8±0.0
PS	1.5±0.0	0.7±0.1	0.9±0.2	0.9±0.0
PE	0.3±0.0	1.4±0.0	1.7±0.0	1.8±0.0
PA/CL	0.5±0.0	0.4±0.0	0.4±0.0	0.8±0.1
Neutral				
MAG	0.4±0.0	0.2±0.1	0.4±0.0	0.9±0.0
DAG	0.5±0.0	0.4±0.0	0.4±0.1	0.8±0.0
C	0.3±0.0	0.3±0.0	0.8±0.2	1.3±0.0
FFA	0.4±0.1	0.5±0.0	0.5±0.1	0.7±0.1
TAG	1.4±0.0	1.7±0.1	3.7±0.3	5.9±0.0
SE	0.6±0.0	0.6±0.1	0.6±0.0	2.1±0.1

Table 3. *Penaeus kerathurus*. Total lipid, total polar lipid, total neutral lipid and neutral lipid contents in midgut gland of females at different stages of sexual maturation. Data are means ± SD of three different samples for each stage, expressed as percentage dry wt. tr: trace (<0.01% dry matter). Abbreviations as in Table 2

Lipid classes	Stages			
	I	II	III	IV
Total				
TL	38.4±1.2	43.6±1.1	39.1±0.9	39.4±1.3
TPL	5.8±0.2	5.7±0.4	5.7±0.4	5.7±0.3
TNL	32.6±0.2	37.8±0.4	33.4±0.4	33.7±0.3
Neutral				
MAG	1.2±0.1	1.1±0.0	0.5±0.1	1.2±0.1
DAG	1.3±0.1	1.3±0.0	1.0±0.0	1.2±0.1
C	tr	tr	tr	tr
FFA	tr	tr	tr	tr
TAG	22.8±0.3	25.9±0.1	25.9±0.4	25.0±0.5
SE	7.4±0.1	9.4±0.4	5.9±0.2	6.3±0.3

decreased between Stages II and III, and then remained constant up to Stage IV (Table 3).

The major lipid classes in the midgut gland were TAG and SE. TAG content increased between Stages I and II and then decreased slightly between Stages II and IV. SE concentrations also increased between Stages I and II, but then decreased abruptly between Stages II and III (Table 3).

Table 4. *Penaeus kerathurus*. Variation in total ovarian lipid fatty acid content (μg fatty acid/mg dry wt) at different stages of sexual maturation. Data are means of three different samples (standard deviations omitted for clarity, but variation generally <5%). PUFA: polyunsaturated fatty acids; HUFA: highly unsaturated fatty acids >20:3. Totals include some minor components (<0.5%) not shown

Fatty acid	Stages			
	I	II	III	IV
Saturated				
14:0	1.47	0.45	3.34	10.28
15:0	0.68	0.45	1.06	1.71
16:0	8.42	8.96	15.28	12.31
17:0	0.31	0.54	1.12	0.90
18:0	5.68	5.87	8.63	10.35
20:0	0.37	0.18	0.44	0.60
22:0	0.01	0.00	0.03	0.13
Total	16.97	16.46	29.92	36.30
Monounsaturated				
16:1n-9	0.89	0.00	5.03	13.03
16:1n-7	2.18	3.18	9.04	8.06
18:1n-9	3.19	3.82	9.34	9.55
18:1n-7	2.06	2.98	7.19	5.27
20:1n-9	1.05	0.68	1.72	1.69
20:1n-7	0.21	0.32	0.90	0.80
22:1n-11	0.26	0.20	0.00	0.00
22:1n-9	0.09	0.00	0.31	0.10
Total	10.51	12.41	34.26	39.91
PUFA (n-6)				
18:2n-6	0.59	0.59	1.29	1.76
18:3n-6	0.03	0.00	0.05	0.10
20:2n-6	0.90	0.35	0.98	1.08
20:3n-6	0.17	0.26	0.37	0.26
20:4n-6	1.80	2.07	3.99	3.93
22:5n-6	0.09	0.11	0.53	0.56
Total	4.97	4.18	8.39	9.40
PUFA (n-3)				
18:3n-3	0.90	0.61	0.27	0.83
18:4n-3	0.14	0.25	0.29	0.48
20:4n-3	0.14	0.09	0.44	0.50
20:5n-3	5.00	6.05	12.65	10.84
22:5n-3	0.51	0.76	2.18	1.73
22:6n-3	2.78	3.81	9.86	8.91
Total (n-3)	10.80	15.84	29.23	27.73
Total PUFA	17.82	21.14	39.36	39.12
HUFA (n-6)	2.80	3.09	7.02	6.32
HUFA (n-3)	9.47	13.53	26.53	23.82

Fatty acid content variations in total lipid and individual lipid classes in ovary and midgut gland

Table 4 shows the variation in ovarian total lipid fatty acid contents during maturation. The major fatty acids were 16:0, 16:1n-9, 16:1n-7, 18:1n-9, 20:5n-3 and 22:6n-3. Total saturated fatty acids, mainly 16:0 and 18:0, were usually deposited between Stages II and III, but 14:0 accumulated mostly between Stages III and IV. Total monounsaturated fatty acids were mainly accumulated during the same period but at higher rates and concentrations, and constituted the predominant individual monoenes 16:1n-9, 16:1n-7 and 18:1n-9. Both saturated

Table 5. *Penaeus kerathurus*. Variations in total lipid fatty acid contents (μg fatty acid/mg dry wt) in midgut gland at different stages of sexual maturation in females. Data are means of three different samples (standard deviations omitted for clarity, but variation generally <5%). Abbreviations as in Table 4. Totals include some minor components (<0.5%) not shown

Fatty acid	Stages			
	I	II	III	IV
Saturated				
14:0	7.09	7.99	6.45	9.63
15:0	3.70	5.28	3.84	6.23
16:0	40.19	45.79	36.57	43.91
17:0	2.19	2.59	2.82	2.72
18:0	21.22	24.13	21.30	15.25
20:0	0.65	0.75	0.48	1.06
22:0	0.32	0.29	0.29	0.17
Total	75.39	86.84	71.78	79.01
Monounsaturated				
16:1n-9	0.00	0.00	0.00	0.00
16:1n-7	16.89	23.81	19.95	20.19
18:1n-9	10.44	12.60	9.02	12.51
18:1n-7	16.75	18.55	14.54	16.88
20:1n-9	7.38	9.19	8.32	10.76
20:1n-7	4.93	6.57	5.33	8.70
22:1n-11	0.71	0.88	1.35	1.15
22:1n-9	1.58	2.59	2.36	1.98
Total	60.93	77.99	62.71	76.48
PUFA (n-6)				
18:2n-6	2.76	2.82	1.41	4.55
18:3n-6	0.42	0.51	0.29	0.00
20:2n-6	3.10	3.74	2.68	4.17
20:3n-6	0.64	0.60	0.38	3.05
20:4n-6	4.97	5.26	5.13	7.69
22:5n-6	1.35	2.09	1.65	2.47
Total (n-6)	20.63	24.41	18.80	27.63
PUFA (n-3)				
18:3n-3	1.47	1.37	1.59	3.23
18:4n-3	1.04	1.44	1.17	1.06
20:4n-3	1.34	1.53	0.74	2.17
20:5n-3	16.92	23.03	19.32	23.89
22:5n-3	4.03	5.57	4.93	5.84
22:6n-3	17.34	23.99	20.37	24.55
Total (n-3)	54.37	69.07	56.17	73.91
Total PUFA	80.79	100.05	81.05	109.41
HUFA (n-6)	9.65	11.44	10.45	17.65
HUfa (n-3)	48.24	62.98	49.76	64.83

Table 6. *Penaeus kerathurus*. Variations in ovarian triacylglycerol fatty acit contents (μg fatty acid/mg dry wt) of females at different stages of sexual maturation. Data are means of three different samples (standard deviations omitted for clarity, but variation generally <5%). Abbreviations as in Table 4. Totals include some minor components (<0.5%) not shown

Fatty acid	Stages			
	I	II	III	IV
Saturated				
14:0	0.31	0.34	0.93	1.01
15:0	0.10	0.13	0.25	0.45
16:0	1.19	1.70	5.67	6.33
17:0	0.07	0.13	0.27	0.33
18:0	0.44	0.48	1.23	1.60
20:0	0.01	0.02	0.07	0.09
22:0	0.01	0.02	0.03	0.04
Total	2.15	2.83	8.47	9.88
Monounsaturated				
16:1n-9	0.00	0.00	0.00	0.00
16:1n-7	0.56	0.77	2.35	3.00
18:1n-9	0.83	1.13	3.37	3.70
18:1n-7	0.43	0.58	1.75	1.47
20:1n-9	0.12	0.18	0.64	0.66
20:1n-7	0.06	0.11	0.43	0.42
22:1n-11	0.02	0.02	0.01	0.07
22:1n-9	0.00	0.00	0.05	0.00
Total	2.08	3.25	9.28	10.10
PUFA (n-6)				
18:2n-6	0.10	0.13	0.39	0.52
18:3n-6	0.01	0.00	0.04	0.04
20:2n-6	0.06	0.09	0.25	0.29
20:3n-6	0.03	0.06	0.08	0.09
20:4n-6	0.20	0.24	0.68	1.08
22:5n-6	0.02	0.04	0.16	0.22
Total (n-6)	0.46	0.60	1.80	2.71
PUFA (n-3)				
18:3n-3	0.09	0.10	0.08	0.05
18:4n-3	0.02	0.03	0.08	0.18
20:4n-3	0.03	0.04	0.11	0.13
20:5n-3	0.53	0.75	2.57	3.16
22:5n-3	0.13	0.16	0.59	0.58
22:6n-3	0.43	0.66	2.45	2.93
Total (n-3)	1.52	1.97	6.94	8.20
Total PUFA	2.12	2.67	9.13	11.43
HUFA (n-6)	0.36	0.47	1.36	1.76
HUFA (n-3)	1.26	1.82	6.23	7.38

and monounsaturated fatty acids also declined in the ovaries between Stages III and IV, but to a lesser degree than in the previous stages. The major polyunsaturated fatty acids (PUFAs) in the total lipids of the ovary were 20:4n-6, 20:5n-3 and 22:6n-3. The concentration of arachidonic acid increased between Stages I and III and then decreased at Stage IV. Docosahexaenoic acid (22:6n-3) although present in smaller concentrations than 20:5n-3, was proportionally stored to a greater extent than 20:5n-3 during Stages I to III. The concentration of fatty acids such as 16:0, 16:1n-7, 18:1n-7, 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3 declined in the final phase of maturation between Stages III and IV.

The modifications of the total lipid fatty acid contents in the midgut gland during maturation are shown in Table 5. Major fatty acids in this organ were 16:0, 18:0, 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, 20:4n-6, 20:5n-3 and 22:6n-3. In general terms, the concentration of all fatty acids had increased by the end of Stage II. Major changes occurred in 16:1n-7, 20:5n-3 and 22:6n-3 (possibly due to ingestion of prey rich in these fatty acids). A noteworthy decline then occurred between Stages II and III. This corresponded with the marked increase in total lipid fatty acid contents in the ovaries during the same period. On the other hand, total lipid fatty acid concentrations of the midgut gland subsequently increased again between Stages III and IV. This was due mainly to total (n-6) highly unsaturated fatty acids (HUFA) and total (n-3) HUFA, although individual fatty acids such as 16:0, 20:5n-3 and 22:6n-3 also displayed large increases.

Table 7. *Penaeus kerathurus*. Variations in ovarian phosphatidylcholine fatty acit contents (μg fatty acid/mg dry wt) of females at different stages of sexual maturation. Data are means of three different samples (standard deviations omitted for clarity, but variation generally <5%). Abbreviations as in Table 4. Totals include some minor components (<0.5%) not shown

Fatty acid	Stages			
	I	II	III	IV
Saturated				
14:0	0.24	0.63	0.53	0.53
15:0	0.15	0.20	0.41	0.35
16:0	2.11	3.37	7.20	5.37
17:0	0.16	0.35	0.61	0.42
18:0	0.98	1.27	3.02	2.91
20:0	0.01	0.14	0.09	0.05
22:0	0.01	0.00	0.02	0.03
Total	3.68	5.98	11.91	9.69
Monounsaturated				
16:1n-9	0.00	0.00	0.00	0.00
16:1n-7	0.91	1.45	3.97	3.26
18:1n-9	1.71	2.57	6.47	4.41
18:1n-7	0.98	1.22	2.05	1.52
20:1n-9	0.25	0.08	0.69	0.53
20:1n-7	0.08	0.06	0.28	0.22
22:1n-11	0.01	0.01	0.00	0.03
22:1n-9	0.00	0.01	0.00	0.03
Total	3.99	5.72	13.72	10.12
PUFA (n-6)				
18:2n-6	0.21	0.34	0.71	0.70
18:3n-6	0.01	0.02	0.05	0.03
20:2n-6	0.17	0.20	0.35	0.08
20:3n-6	0.03	0.04	0.08	0.01
20:4n-6	0.80	0.96	1.96	1.65
22:5n-6	0.05	0.08	0.26	0.21
Total (n-6)	1.35	2.01	4.01	2.96
PUFA (n-3)				
18:3n-3	0.08	0.04	0.19	0.07
18:4n-3	0.04	0.23	0.09	0.09
20:4n-3	0.05	0.06	0.16	0.11
20:5n-3	2.48	3.08	6.95	4.60
22:5n-3	0.24	0.31	0.98	0.65
22:6n-3	1.29	1.65	4.69	3.20
Total (n-3)	4.71	5.69	14.09	10.39
Total PUFA	6.16	7.90	18.71	13.89
HUFA (n-6)	1.08	1.31	3.03	2.33
HUFA (n-3)	4.26	5.25	13.22	9.37

Table 8. *Penaeus kerathurus*. Variations in ovarian phosphatidylethanolamine fatty acid contents (μg fatty acid/mg dry wt) of females at different stages of sexual maturation. Data are means of three different samples (standard deviations omitted for clarity, but variation generally <5%). Abbreviations as in Table 4. Totals include some minor components (<0.5%) not shown

Fatty acid	Stages			
	I	II	III	IV
Saturated				
14:0	0.13	0.55	0.53	0.86
15:0	0.01	0.41	0.35	0.45
16:0	0.04	0.36	0.25	0.16
17:0	0.01	0.07	0.09	0.06
18:0	0.25	0.61	0.76	0.87
20:0	0.01	0.01	0.01	0.03
22:0	0.01	0.00	0.00	0.01
Total	0.47	2.03	2.02	2.46
Monounsaturated				
16:1n-9	0.01	0.28	0.80	0.66
16:1n-7	0.00	0.18	0.47	0.25
18:1n-9	0.07	0.35	0.49	0.45
18:1n-7	0.05	0.34	0.49	0.42
20:1n-9	0.01	0.06	0.10	0.09
20:1n-7	0.01	0.02	0.05	0.05
22:1n-11	0.01	0.00	0.00	0.01
22:1n-9	0.00	0.01	0.00	0.02
Total	0.22	1.35	2.50	2.12
PUFA (n-6)				
18:2n-6	0.02	0.07	0.04	0.13
18:3n-6	0.00	0.01	0.00	0.01
20:2n-6	0.06	0.04	0.06	0.04
20:3n-6	0.03	0.01	0.00	0.02
20:4n-6	0.03	0.45	0.73	0.55
22:5n-6	0.00	0.04	0.13	0.10
Total (n-6)	0.19	0.71	1.12	1.00
PUFA (n-3)				
18:3n-3	0.07	0.06	0.08	0.18
18:4n-3	0.01	0.01	0.01	0.03
20:4n-3	0.01	0.01	0.00	0.01
20:5n-3	0.05	1.54	2.62	1.62
22:5n-3	0.01	0.17	0.43	0.28
22:6n-3	0.09	1.11	2.07	1.45
Total (n-3)	0.29	3.64	6.31	4.93
Total PUFA	0.50	4.46	7.51	6.00
HUFA (n-6)	0.07	0.64	1.17	0.85
HUFA (n-3)	0.19	2.93	5.42	3.91

The variations of TAG fatty acid contents in the ovaries of *Penaeus kerathurus* during sexual maturation, are shown in Table 6. The fatty acids of this class were dominated by 16:0, 16:1n-7, 18:1n-9, 20:5n-3 and 22:6n-3. The amounts of these fatty acids increased by between 5.31 and 6.81 times between Stages I and IV, although the most significant increases occurred between Stages II and III, when the concentration of the major fatty acid was enhanced by 3.05 to 3.71 times.

The evolution of PC fatty acid content of ovaries is indicated in Table 7. The most abundant fatty acids in this fraction were 16:0, 18:0, 16:1n-7, 18:1n-9, 20:4n-6, 20:5n-3 and 22:6n-3, and by the end of Stage III their concentrations had increased by 2.45 to 4.36 times over the initial levels. However, from Stages III to IV a certain decline was observed, with decreases ranging from 3.64 to 33.8%. The contents of 18:1n-9, 20:5n-3 and 22:6n-3 decreased during this period.

The modifications of the fatty acid contents corresponding to the PE fraction through ovary maturation are shown in Table 8. The main feature was the large increase in fatty acid content from Stages I through III. Total saturates did not increase between Stages II and III, whereas the amounts of monoenes and polyunsaturates did. In contrast, between Stages III and IV, total saturated fatty acid contents increased while total monounsaturated and polyunsaturated fractions decreased.

The midgut gland stored large amounts of TAG and SE, whereas total PL contents remained constant during maturation. Therefore, the fatty acid composition of the

Table 9. *Penaeus kerathurus*. Variations in triacylglycerol fatty acid contents (μg fatty acid/mg dry wt) in midgut gland of females at different stages of sexual maturation. Data are means of three different samples (standard deviations omitted for clarity, but variation generally <5%). Abbreviations as in Table 4. Totals include some minor components (<0.5%) not shown

Fatty acid	Stages			
	I	II	III	IV
Saturated				
14:0	6.42	9.53	6.63	10.60
15:0	2.30	2.78	2.05	2.84
16:0	21.99	26.30	24.24	30.45
17:0	1.34	1.54	1.88	1.72
18:0	7.04	8.67	7.12	9.55
20:0	0.48	0.36	0.49	0.42
22:0	0.22	0.25	0.34	0.07
Total	39.81	49.45	42.77	55.67
Monounsaturated				
16:1n-9	0.00	0.00	0.00	0.00
16:1n-7	8.88	12.50	13.08	13.51
18:1n-9	4.02	6.37	3.39	5.92
18:1n-7	4.66	6.56	4.04	6.75
20:1n-9	6.09	2.88	7.33	8.10
20:1n-7	5.46	4.71	5.70	7.29
22:1n-11	1.37	1.85	0.13	0.08
22:1n-9	0.80	1.26	0.17	0.14
Total	34.39	39.56	36.48	42.13
PUFA (n-6)				
18:2n-6	1.60	2.35	0.93	1.64
18:3n-6	0.48	0.54	0.45	0.98
20:2n-6	2.30	2.35	2.59	2.47
20:3n-6	0.66	0.52	0.77	0.40
20:4n-6	2.81	3.44	3.65	4.91
22:5n-6	0.90	1.18	1.08	1.62
Total (n-6)	10.28	12.19	12.59	13.71
PUFA (n-3)				
18:3n-3	1.39	1.50	1.45	1.03
18:4n-3	0.65	0.76	0.69	1.15
20:4n-3	0.84	0.91	0.63	0.66
20:5n-3	10.75	15.11	12.88	16.06
22:5n-3	2.40	3.17	2.92	3.20
22:6n-3	10.97	14.72	13.14	16.22
Total (n-3)	30.84	40.39	37.08	43.97
Total PUFA	43.50	55.28	53.25	61.62
HUFA (n-6)	5.88	7.14	8.20	8.67
HUFA (n-6)	27.48	36.56	32.61	39.81

total PL did not vary through this interval (data not shown). The variations of TAG fatty acid contents of the midgut gland are given in Table 9. Practically all fatty acids increased in concentration between Stages I and IV, but most, mainly 14:0, 16:0, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 20:5n-3 and 22:6n-3, declined between Stages II and III.

Discussion

The biometric data for *Penaeus kerathurus* (Table 1) agree with those given for this species by Rodríguez (1985) and the GI values for each stage agree with the GI values and stages described for wild *P. japonicus* by Teshima and Kanazawa (1983).

An increase in the lipid concentration of the ovaries with a concurrent decrease in the hepatopancreatic lipids has been noted by Teshima and Kanazawa (1983) for wild *Penaeus japonicus* females during maturation. A similar pattern was exhibited by *P. kerathurus* in the present study. During the period of gonadal development, the total lipid contents of the ovaries and midgut gland of *P. kerathurus* females were higher than the lipid contents of the same organs of wild-caught females of *P. monodon* (Millanema and Pascual 1990). The ovaries of *P. kerathurus* contained much higher quantities of PL than of NL from Stages I through III, but equal amounts were found at Stage IV; this contrasts with the results found for *Charismus antarcticus* by Clarke (1977) and for *P. japonicus* by Guary et al. (1974) and Teshima et al. (1989). Nevertheless, PL concentrations predominate over NL concentrations in ovaries of shrimps such as *P. duorarum* (Gehring 1974), *P. indicus* (Galois 1984) and *Pleoticus muelleri* (Jeckel et al. 1989). Phospholipids were largely responsible for the increases in ovarian lipids in *Penaeus kerathurus*, which conforms with data reported for developing ovaries of *P. monodon* (Millanema and Pascual 1990). In contrast, in the midgut gland of *P. kerathurus*, which contained higher mean levels of lipids, NL prevailed over PL (Tables 2 and 3). A similar result was reported by Millanema and Pascual for the midgut gland of *P. monodon* during maturation.

Phospholipids are considered to be principal lipid components of the haemolymph and tissues of crustaceans, except in the midgut gland where it is well established that neutral lipids (particularly TAG) represent the bulk of total lipids (Chapelle 1977). PC and PE are the major circulating lipids in the crustacean haemolymph (Gilbert and O'Connor 1970, Allen 1972, Lee and Puppione 1978), whereas ingested neutral lipids are enzymatically cleaved (mainly by TAG lipases) to either α,β-diglycerides or β-monoglycerides, both of which are probably converted to phospholipids by the absorptive cells of the midgut gland (Chang and O'Connor 1983). It seems evident that the majority of the neutral lipids ingested by *Penaeus kerathurus* females are converted to polar lipids in the midgut gland and transported to the developing ovaries for use as constituent and reserve lipids during sexual maturation.

Total ovarian lipids in *Penaeus kerathurus* reached a maximum level at Stage IV, as reported for *P. japonicus* by Teshima and Kanazawa (1983). In *P. duorarum* (Gehring 1974) and *P. monodon* (Millanema and Pascual 1990), however, the highest mean lipid levels in ovaries occurred at Stage III (late maturing ovaries). The PC concentration in the ovaries of *P. kerathurus* decreased slightly between Stages III and IV (Table 2); it is unclear if this was due to interconversion into other polar lipid classes or conversion into other compounds associated with the rod-like bodies that first appear at Stage IV (King 1948, Gehring 1974, Rodríguez 1985). On the other hand, PE did show an increase between Stages I and II, similar to that found for *P. duorarum* by Gehring. Nevertheless, from the point of view of PL reserves, the sum of

the PL fractions is more relevant than the changes in the concentrations of the individual PL classes, since their biosynthetic pathways are closely related and interconversion is easily carried out (Chapelle 1986).

In the midgut gland, the total PL concentration remained constant during ovarian maturation (ca. 5.7% of dry wt) and TAG and SE were the predominant lipid classes (Table 3). The main roles of lipid storage in the midgut gland are as reserves for vitellogenesis and moulting (Dall 1981). Since in decapod crustaceans the main lipid source during vitellogenesis seems to be food (Galois 1984), and since TAG is the predominant lipid class in the midgut gland and also the second in importance in the ovary, it is clear that abundant and continuous feeding during maturation of *Penaeus kerathurus* plays a very important role in supplying lipids during the process of formation and maturation of ovaries (see Sargent 1976). In consequence, it is quite feasible that *P. kerathurus* could have a higher dietary lipid requirement during maturation. The lipid component in the ovary increases during development, and the midgut gland has a limited storage capability, as illustrated for *P. stylirostris* by Bray et al. (1990). The TAG of the midgut gland may be transported to the ovaries as lipoproteins rich in phospholipids, after having being transformed to phospholipids within the midgut gland (Teshima and Kanazawa 1983). As described by Clarke (1982), during maturation the digestive gland of *P. kerathurus* females is in a state of flux, with food lipids being absorbed and egg lipids being produced, and the midgut gland serves more as a metabolic nucleus reconverting incoming lipids than as a reservoir or source of yolk. The midgut gland also seems to be the principal site of cholesterol absorption and storage, mainly as SE (see Table 3). However, the concentration of free sterols is higher in the ovaries (Table 2) and its presence in the oocyte may indicate an essential role during vitellogenesis (Blanchet-Tournier 1982). Besides its role as a membrane constituent (Kanazawa and Teshima 1971), the cholesterol in the egg yolk is the precursor of steroid hormones in crustaceans, and it is used by the larvae during the naupliar stage. The ovary is presumably the major site of cholesterol metabolism followed by the midgut gland (see Kanazawa et al. 1988).

It has been assumed that polyunsaturated fatty acids are involved to some extent in the capacity of the reproductive processes of shrimps such as *Penaeus stylirrostris* (Brown et al. 1979), *P. setiferus* (Lawrence et al. 1980) or *P. vannamei* (Cahu and Quazuguel 1989). Moreover, eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) have been shown to be major components of phospholipids belonging to eye membranes of the prawn *Pandalus borealis* (Bell and Dick 1990), indicating that these fatty acids may play an important role in neural tissues of marine crustaceans. In *Penaeus kerathurus*, ~65% of the fatty acids of the total ovarian lipids are conveyed to eggs and embryos during spawning; the transferred concentrations of 20:5n-3 and 22:6n-3 comprise 4 and 4.5% of the total ovary lipids, respectively (Mourente et al. 1990). In the present study, the lipids of the midgut gland contained very high levels of 16:0, monoenes such as 16:1n-7 and 18:1n-9, and HUFA such as 20:4n-6, 20:5n-3 and 22:6n-3. The large increase in these fatty acids in the ovaries of *P. kerathurus* during maturation reflects the fatty acid requirement of this tissue and that required for transfer to the developing embryos (Tables 4 and 5). The TAG fatty acid contents increased with ovarian development (Table 6), forming an energy reserve and a temporary reservoir for PUFAs for future embryonic development (Napolitano et al. 1988). The decreases in the fatty acid contents of TAG in the midgut gland between Stages II and III (Table 9) probably result from the transfer of these fatty acids to phospholipids in the ovaries (Chang and O'Connor 1983). These fatty acids, stored as TAG in the midgut gland, may also play an important "reserve role" in the case of prolonged starvation before or during maturation or moulting (Dall 1981). The increase in the concentration of PUFAs such as 20:4n-6, 20:5n-3 and 22:6n-3 in the TAG fractions of both ovary and midgut gland (Tables 6 and 9), would seem to reinforce the theory that long-chain fatty acids are necessary for vitellogenesis of penaeids (Middleditch et al. 1980). Arachidonic and eicosapentaenoic acids are precursors to prostaglandins and may also have an important role in reproduction (Middleditch et al. 1979) or in inducing hatching, as shown by Holland et al. (1985) for the eggs of the barnacle *Balanus balanoides*. The major fatty acids in ovarian PC were 16:0, 18:1n-9, 20:5n-3 and 22:6n-3, and their concentrations increased between Stages I and III (Table 7). The PE was dominated by these same acids, but displayed a more unsaturated pattern and a noteworthy increase of (n-3) HUFA between Stages I and II, indicating the importance of the presence of high levels of these fatty acids in this fraction during the early formation of biomembranes in the developing ovary of *P. kerathurus* (Table 8). In the ovaries, both PC and PE, were affected by a notable decrease in PUFA concentrations at Stage IV (Tables 7 and 8), as reported for *P. duorarum* (Gehring 1974) and *P. monodon* (Millanema and Pascual 1990). This might be due to the conversion of these fatty acids into other compounds or their use as energy sources.

Although the data presented in this study help to elucidate the role of the different lipid fractions in female *Penaeus kerathurus* during maturation, further work will be necessary to elaborate the understanding of lipid metabolism during ovarian development in this species.

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