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Changes in lipid class and fatty acid contents in the ovary and midgut gland of the female fiddler crab *Uca tangeri* (Decapoda, Ocypodiadae) during maturation

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Abstract Changes in biochemical composition, lipid class and fatty acid contents were studied in the ovaries and midgut glands of the fiddler crabs *Uca tangeri* Eydoux during maturation. Wild females were caught during spring and early summer of 1992 in the Bay of Cádiz (southwest Spain), near the mouth of the San Pedro river. Protein and total lipid contents in the ovaries increased significantly from Stages III to IV, at the expense of total carbohydrate, which showed a large decrease during the same period. In the midgut gland, the protein content did not present any significant variation, whereas total lipids and total carbohydrates presented opposite up and down trends during maturation. In the ovary, total polar lipids increased significantly during the final phase of maturation (Stages III to IV), mainly due to the significant contribution of the phosphatidylcholine and phosphatidylethanolamine fractions. In contrast, total neutral lipids showed an upward trend throughout the whole maturation period, mainly due to significant increases of the triacylglycerol fraction. In the midgut gland, total polar lipids (mainly phosphatidylcholine) and total neutral lipids (mainly triacylglycerol) presented significant decreases from Stages II to III, the phase which preceded major increases in both polar and neutral lipids in the ovaries. Cholesterol content did not vary during maturation in either organ, in the ovary or midgut gland. Major fatty acids in the ovaries [16:0, 16:1 (n-7), 18:1 (n-9), 18:1 (n-7), 18:2 (n-6), 18:3 (n-3), 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3)] did, however, accumulate significantly at later stages of maturation. It is noteworthy that arachidonic acid [20:4 (n-6)] content remained constant during all stages of maturation but decreased significantly in total polar lipids in the later phases of maturation. In contrast, eicosapentaenoic acid [20:5 (n-3)] increased significantly in all lipid fractions in the later stages, and docosahexaenoic acid [22:6 (n-3)] remained constant in the polar lipids and increased during later stages in the triacylglycerol fraction. Major fatty acids in the midgut gland lipids showed significant decreases from Stages II to III, just before the final period of maturation.

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Introduction

Lipids play several important roles in the biochemistry, metabolism and reproduction of decapod crustaceans (Morris 1973; Gehring 1974; Middleditch et al. 1980; Read and Caulton 1980; Galois 1984; Clarke et al. 1985; Lautier and Lagarrigue 1988; Teshima et al. 1988 a, b; Castille and Lawrence 1989; Jeckel et al. 1989; Bray et al. 1990; Mourente et al. 1990; Mourente and Rodríguez 1991; Alava et al. 1993; Xu et al. 1993). Neutral lipids, particularly triacylglycerides (TAG), are a major energy source and the predominant form of energy storage (Clarke 1982; Teshima and Kanazawa 1983; Harrison 1990). Phospholipids and sterols perform important functions as essential constituents of biological cell membranes, affecting their structural and physiological properties. Phospholipids are also the major transport form of lipids in the hemolymph (Teshima and Kanazawa 1980; Lee and Puppione 1988; Harrison 1990; Alava et al. 1993). In decapods, the midgut gland is the main lipid storage and processing organ (Vogt et al. 1985; Harrison 1990), although during maturation the ovary becomes an additional centre for lipid metabolism, including lipogenesis (Harrison 1990). However, the midgut gland lipid reserves only contribute partially to vitellogenesis, and dietary lipid reserves must be processed rapidly through this organ and exported to the ovary during maturation (Galois 1984; Harrison 1990; Mourente and

Table 2 *Uca tangeri*. Changes in gross composition and lipid class contents (dry weight percentage) in ovary at different stages of maturation. 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

	Stage of maturation			
	I	II	III	IV
Dry weight	20.5 ± 0.6^{a}	32.3 ± 2.5^{b}	48.1 ± 1.5^{c}	53.1 ± 1.9^{d}
Protein	59.1 ± 0.9^{a}	59.7 ± 2.8^{ab}	53.6 ± 2.7^{a}	65.7 ± 1.5^{b}
Lipid	10.6 ± 0.1^{a}	12.3 ± 0.1^{a}	14.7 ± 1.5^{a}	19.7 ± 2.2^{b}
Carbohydrate	18.5 ± 0.8^{a}	16.9 ± 0.5^{a}	29.5 ± 1.8^{b}	9.0 ± 1.1^{c}
Ash	11.8 ± 0.5^{a}	11.1 ± 0.7^{a}	5.7 ± 0.4^{b}	5.6 ± 0.2^{b}
Total polar lipids	5.6 ± 0.1^{a}	5.7 ± 0.6^{ab}	5.1 ± 0.2^{b}	7.0 ± 0.5^{c}
Phosphatidylcholine	2.5 ± 0.1^{a}	2.8 ± 0.4^{ab}	3.0 ± 0.1^{b}	4.4 ± 0.3^{c}
Phosphatidylethanolamine	1.4 ± 0.0^{a}	1.5 ± 0.1^{a}	1.3 ± 0.0^{a}	1.8 ± 0.1^{b}
Phosphatidylserine	0.4 ± 0.0^{a}	0.3 ± 0.0^{b}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}
Phosphatidylinositol	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}
Phosphatidic acid/cardiolipin	0.3 ± 0.0^{a}	0.2 ± 0.0^{b}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}
Sphingomyelin	0.4 ± 0.0^{a}	0.3 ± 0.0^{b}	0.2 ± 0.0^{c}	0.2 ± 0.0^{c}
Pigments	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1
Total neutral lipids	4.9 ± 0.1^{a}	6.5 ± 0.6^{b}	9.2 ± 0.2^{c}	13.1 ± 0.4^{d}
Cholesterol	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.2 ± 0.6
Free fatty acid	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.1
Triacylglycerol	3.3 ± 0.2^{a}	4.7 ± 0.4^{b}	7.0 ± 0.0^{c}	10.9 ± 0.3^{d}
Steryl ester	0.3 ± 0.0^{a}	0.5 ± 0.1^{b}	0.6 ± 0.1^{b}	0.8 ± 0.0^{c}

Materials

All solvents were analar grade and supplied by Merck, Darmstadt (Germany). Potassium bicarbonate, potassium chloride, cupric acetate, BHT and nonadecanoic acid (>90% 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 ortho-phosphoric acid were purchased from Fluka Chemicals Co. Ltd.

Results

Biochemical compositions and lipid class contents in the ovary and midgut gland

No significant differences were found among carapace length, carapace width or total weights of females belonging to different maturational stages. Ovarian wet weight and GI significantly increased during maturation (Table 1).

The variations in gross composition and lipid class contents in the ovary during maturation are presented in Table 2. Ovary dry weight increased significantly by 36.5 and 32.8% from Stages I to II and Stages II to III, respectively, and increased by only 9.4% from Stages III to IV. Protein and lipid contents showed no significant increases from Stages I to III, but they increased significantly from Stages III to IV. In contrast, total carbohydrate presented a significant increase between Stages II and III, but showed a large decrease between Stages III and IV. Total polar lipid content showed a significant increase (by 27.1%) from Stages III to IV only, mainly due to significant increases in major polar lipid classes, phosphatidylcholine (PC) and phosphatidylethanolamine (PE). In contrast, minor polar lipid classes such as phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid/cardiolipin (PA/CL) and sphingomyelin (SM) decreased throughout the maturation period. Total neutral lipid content increased significantly during maturation (by 24.6, 29.3, and 29.8%, respectively, from Stages I to IV) due to increases in the major neutral lipid classes, the triacylglycerol and steryl ester fractions, respectively.

Changes in gross composition and lipid class contents in the midgut gland are shown in Table 3. Dry weight showed a significant increase from Stages III to IV, mainly due to lipid contribution. Protein content did not present any variation. In comparison, total lipid content increased significantly from Stages I to II, then decreased from Stages II to III and increased again from Stages III to IV, whereas total carbohydrate content showed an up and down trend opposite to that of total lipids. Ash content decreased significantly from Stages II to III. Total polar lipids and PC exhibited significant decreases (by 37.5 and 33.3%, respectively) from Stages II to III. The rest of polar lipid classes did not present any significant variations in their respective contents during maturation. Total neutral lipid content (primarily triacylglycerol) in the midgut gland was more abundant than total polar lipids and showed quantitative variations throughout maturation similar to those presented by total lipids. Similar up and down trends were also shown by other neutral lipid classes such as free fatty acids (FFA) and SE fractions.

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

Table 4 shows the variation in ovarian total lipid fatty acid contents during maturation. The predominant fatty acids were 16:0, 16:1 (n-7), 18:1 (n-9), 18:1 (n-7), 18:2 (n-6), 18:3 (n-3), 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3).

Table 3 *Uca tangeri.* Changes in gross composition and lipid class contents (dry weight percentage) in midgut gland at different stages of maturation. 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. (*tr* trace)

	Stage of maturation			
	I	II	III	IV
Dry weight	30.7 ± 2.4^{a}	31.1 ± 7.7^{a}	38.1 ± 5.9^{ab}	44.8 ± 5.3^{b}
Protein	38.3 ± 4.4	37.2 ± 2.8	36.9 ± 2.9	38.3 ± 3.7
Lipid	9.0 ± 0.7^{a}	15.9 ± 0.5^{b}	12.0 ± 0.7^{c}	19.8 ± 0.3^{d}
Carbohydrate	38.0 ± 1.4^{a}	31.2 ± 0.9^{b}	42.6 ± 3.4^{a}	34.4 ± 2.7^{ab}
Ash	14.7 ± 1.2^{a}	15.7 ± 1.5^{a}	8.5 ± 1.3^{b}	7.5 ± 0.3^{b}
Total polar lipids	2.6 ± 0.1^{a}	3.2 ± 0.3^{a}	2.0 ± 0.1^{b}	2.3 ± 0.1^{ab}
Phosphatidylcholine	0.8 ± 0.0^{a}	0.9 ± 0.1^{a}	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}
Phosphatidylethanolamine	0.8 ± 0.0	0.9 ± 0.1	0.6 ± 0.1	0.8 ± 0.0
Phosphatidylserine	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Phosphatidylinositol	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Phosphatidic acid/cardiolipin	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Sphingomyelin	0.1 ± 0.0	0.1 ± 0.0	tr	tr
Pigments	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
Unknown	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Total neutral lipids	6.1 ± 0.0^{a}	12.5 ± 0.2^{b}	9.8 ± 0.1^{c}	17.2 ± 0.1^{d}
Cholesterol	0.9 ± 0.0	1.2 ± 0.1	0.8 ± 0.1	1.2 ± 0.0
Free fatty acid	1.5 ± 0.0^{a}	2.1 ± 0.0^{b}	1.4 ± 0.1^{a}	2.4 ± 0.2^{b}
Triacylglycerol	3.0 ± 0.0^{a}	7.6 ± 0.3^{b}	6.5 ± 0.2^{c}	12.1 ± 0.3^{d}
Steryl ester	0.7 ± 0.0^{a}	1.6 ± 0.1^{b}	1.1 ± 0.1^{c}	1.6 ± 0.0^{b}

Table 4 *Uca tangeri.* Variations in total ovarian lipid fatty acid contents (μg fatty acid mg^{-1} dry wt) at different stages of sexual maturation. Data are means ± SD ($n = 3$). SD = 0.0 implies an SD of <0.05. (*tr* trace < 0.1. *HUFA* highly unsaturated fatty acids > 20 : 3). Totals include some minor components not shown. Values within a given row not bearing the same superscript letter are significantly different ($P < 0.05$). If no superscript appears, values are not different

Fatty acid	Stage of maturation			
	I	II	III	IV
14 : 0	1.7 ± 0.1^{a}	2.1 ± 0.1^{ab}	2.5 ± 0.2^{b}	3.5 ± 0.2^{c}
15 : 0	2.4 ± 0.4	2.5 ± 0.2	2.6 ± 0.0	3.5 ± 0.7
16 : 0	20.4 ± 2.5^{a}	25.1 ± 0.4^{a}	28.7 ± 0.6^{b}	38.8 ± 0.9^{c}
17 : 0	1.2 ± 0.1^{a}	1.3 ± 0.0^{a}	1.9 ± 0.2^{b}	2.5 ± 0.2^{c}
18 : 0	4.9 ± 0.4^{a}	4.6 ± 0.0^{a}	6.2 ± 0.0^{b}	10.0 ± 0.2^{c}
20 : 0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
Total saturated	31.4 ± 3.7^{a}	36.4 ± 0.2^{a}	42.5 ± 0.7^{b}	59.1 ± 2.1^{c}
16 : 1 (n-7)	12.9 ± 1.3^{a}	16.7 ± 0.3^{b}	21.4 ± 1.3^{c}	33.4 ± 1.3^{d}
18 : 1 (n-9)	8.3 ± 0.7^{a}	8.4 ± 0.3^{a}	8.9 ± 0.2 a	13.9 ± 0.6^{b}
18 : 1 (n-7)	4.0 ± 0.3^{a}	4.3 ± 0.1^{a}	5.1 ± 0.1^{b}	7.4 ± 0.5^{c}
20 : 1 (n-9)	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
20 : 1 (n-7)	1.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.6 ± 0.2
22 : 1 (n-11)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
22 : 1 (n-9)	0.4 ± 0.2	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
Total monoenes	27.4 ± 2.6^{a}	30.9 ± 0.3^{a}	37.2 ± 1.1^{b}	57.3 ± 3.1^{c}
16 : 2	1.2 ± 0.1^{a}	2.0 ± 0.0^{b}	2.7 ± 0.1^{c}	3.5 ± 0.2^{d}
16 : 3	4.1 ± 0.5	4.0 ± 0.3	4.5 ± 0.2	5.7 ± 1.1
16 : 4	0.6 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
18 : 2 (n-6)	2.5 ± 0.2^{a}	3.1 ± 0.1^{ab}	3.8 ± 0.5^{b}	5.0 ± 0.3^{c}
18 : 3 (n-3)	1.2 ± 0.0^{a}	1.6 ± 0.0^{a}	3.0 ± 0.6^{b}	5.6 ± 0.4^{c}
18 : 4 (n-3)	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}	0.7 ± 0.0^{b}	1.1 ± 0.1^{b}
20 : 2 (n-6)	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
20 : 3 (n-6)	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
20 : 3 (n-3)	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	0.4 ± 0.0^{b}
20 : 4 (n-6)	6.2 ± 0.6	6.2 ± 0.3	6.3 ± 0.5	5.6 ± 1.3
20 : 4 (n-3)	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}	0.5 ± 0.0^{b}
20 : 5 (n-3)	9.9 ± 0.9^{a}	10.6 ± 0.2^{a}	14.9 ± 0.9^{b}	18.7 ± 0.7^{c}
22 : 5 (n-6)	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.5 ± 0.0^{ab}	0.6 ± 0.0^{b}
22 : 5 (n-3)	0.5 ± 0.0^{a}	0.6 ± 0.0^{ab}	0.8 ± 0.0^{bc}	0.9 ± 0.0^{c}
22 : 6 (n-3)	3.2 ± 0.4^{a}	3.8 ± 0.2^{ab}	4.9 ± 0.3^{c}	4.7 ± 1.1^{bc}
HUFA (n-6)	7.4 ± 0.8	7.5 ± 0.3	8.1 ± 0.5	8.6 ± 1.2
HUFA (n-3)	14.3 ± 1.3^{a}	15.6 ± 0.3^{a}	21.8 ± 1.1^{b}	25.6 ± 1.6^{b}

Table 5 *Uca tangeri*. Variations in midgut gland total lipid fatty acid contents (μg fatty acid mg^{-1} dry wt) at different stages of sexual maturation. Data are means ± SD ($n=3$). SD = 0.0 implies an SD of <0.05. (*tr* trace < 0.1. *HUFA* highly unsaturated fatty acids > 20:3). Totals include some minor components not shown. Values within a given row not bearing the same superscript letter are significantly different ($P<0.05$). If no superscript appears, values are not different

Fatty acid	Stage of maturation			
	I	II	III	IV
14:0	1.8±0.0[a]	5.6±0.2[b]	5.0±0.1[b]	7.7±1.3[c]
15:0	1.3±0.0[a]	5.7±0.1[b]	2.8±0.3[c]	5.9±1.4[b]
16:0	15.6±0.1[a]	33.4±0.9[b]	26.2±0.6[c]	39.4±0.9[d]
17:0	0.8±0.0[a]	1.3±0.1[b]	1.2±0.1[b]	1.4±0.2[b]
18:0	4.0±0.0[a]	5.4±0.3[b]	4.2±0.3[a]	6.3±1.2[b]
20:0	0.2±0.0	0.5±0.0	0.3±0.0	0.5±0.1
Total saturated	24.1±0.1[a]	52.3±1.6[b]	40.3±0.8[c]	61.9±4.8[b]
16:1 (n-7)	11.9±0.3[a]	25.1±0.6[b]	19.1±0.7[c]	27.5±3.7[b]
18:1 (n-9)	4.0±0.1[a]	7.4±0.3[b]	4.4±0.2[a]	9.0±1.6[b]
18:1 (n-7)	3.0±0.0[a]	4.9±0.3[b]	5.1±0.1[b]	6.3±1.0[b]
20:1 (n-9)	0.1±0.0[a]	0.3±0.0[b]	0.2±0.1[b]	0.3±0.1[b]
20:1 (n-7)	0.8±0.1[a]	2.1±0.1[b]	0.8±0.1[a]	1.8±0.4[b]
22:1 (n-11)	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.1
22:1 (n-9)	0.1±0.0[a]	0.6±0.0[b]	0.2±0.0[a]	0.2±0.0[a]
Total monoenes	20.1±0.2[a]	40.5±1.0[b]	29.9±0.9[c]	45.4±1.7[b]
16:2	3.1±0.1[a]	8.7±0.2[b]	5.2±0.2[c]	8.2±0.9[b]
16:3	2.2±0.1[a]	5.8±0.3[b]	2.8±0.2[a]	5.8±1.1[b]
16:4	0.3±0.0[a]	0.7±0.0[b]	0.7±0.1[b]	1.1±0.3[b]
18:2 (n-6)	1.7±0.1[a]	2.7±0.1[b]	2.6±0.1[b]	3.2±0.6[b]
18:3 (n-3)	1.2±0.1[a]	2.9±0.1[b]	4.0±0.2[c]	5.9±0.5[d]
18:4 (n-3)	0.3±0.0[a]	1.1±0.4[b]	0.9±0.1[b]	1.5±0.5[b]
20:2 (n-6)	0.3±0.1	0.4±0.1	0.3±0.0	0.4±0.0
20:3 (n-6)	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
20:3 (n-3)	0.1±0.0[a]	0.3±0.0[b]	0.3±0.1[b]	0.6±0.2[b]
20:4 (n-6)	4.0±0.1[a]	4.2±0.2[a]	2.6±0.1[b]	2.6±0.4[b]
20:4 (n-3)	0.2±0.0[a]	0.5±0.0[b]	0.3±0.0[a]	0.5±0.2[b]
20:5 (n-3)	5.8±0.2[a]	10.3±0.3[b]	7.0±0.3[c]	10.4±1.8[b]
22:5 (n-6)	0.2±0.0	0.3±0.1	0.3±0.0	0.2±0.0
22:5 (n-3)	0.3±0.0[a]	0.5±0.0[b]	0.4±0.0[ab]	0.5±0.1[b]
22:6 (n-3)	2.4±0.0[a]	3.4±0.2[b]	1.5±0.1[c]	2.0±0.5[ac]
HUFA (n-6)	5.2±0.1[a]	6.3±0.6[a]	4.3±0.2[b]	4.8±0.7[ab]
HUFA (n-3)	8.9±0.1[a]	15.4±0.5[b]	9.8±0.4[a]	14.4±3.8[b]

Total saturated fatty acids, primarily 16:0 and 18:0, increased significantly from Stages II to III (by 12.5 and 25.8%, respectively) and from Stages III to IV (by 26 and 38%, respectively). Total monounsaturated fatty acids were mainly accumulated during the same period, but major individual monoenes increased following different patterns. The content of 16:1 (n-7) increased significantly throughout all maturation stages, whereas the second major monoene, 18:1 (n-9), only increased from Stages III to IV. The major PUFAs in total lipids from the ovary were 18:2 (n-6), 18:3 (n-3), 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3). Arachidonic acid [AA, 20:4 (n-6)] content remained constant during all maturation stages, EPA showed significant increases from Stages II to III and from Stages III to IV, and DHA only presented a significant increase from Stages II to III.

Modifications of the total lipid fatty acid content in the midgut gland during maturation are shown in Table 5. Total saturated fatty acids, mainly 16:0, presented a significant increase from Stages I to II, decreased significantly from Stages II to III, and a new significant increase was observed from Stages III to IV. The second major saturated fatty acid, 18:0, presented a similar trend but the initial increase, from Stages I to II, was not so marked. Total monounsaturated fatty acids [primarily 16:1 (n-7) and 18:1 (n-9)] also showed a similar trend, presenting significant ups and downs in similar proportions. Major PUFAs were 16:2, 16:3, 18:2 (n-6), 18:3 (n-3), 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3). C_{16} polyenes presented significant increases from Stages I to II, followed by significant decreases from Stages II to III, and then increased significantly from Stages III to IV. In contrast, C_{18} PUFAs showed significant increases throughout maturation. AA (arachidonic acid) exhibited a significant decrease from Stages II to III. EPA increased significantly from Stages I to II, then decreased significantly from Stages II to III, to increase again from Stages III to IV. DHA showed the lowest contents and a similar behaviour throughout maturation to the C_{20} PUFAs.

Variations of fatty acid contents from total polar lipids in the ovaries of *Uca tangeri* during sexual maturation are shown in Table 6. Fatty acids belonging to polar lipid classes were dominated by 16:0, 18:0, 16:1 (n-7), 18:1 (n-9), 18:1 (n-7), 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3). The amounts of most of these fatty acids [with the exceptions of 16:1 (n-7), 20:4 (n-6) and 22:6 (n-3)] remained

Table 6 *Uca tangeri*. Variations in ovarian total polar lipid fatty acid contents (μg fatty acid mg^{-1} dry wt) at different stages of sexual maturation. Data are means ± SD ($n=3$). SD = 0.0 implies an SD of <0.05. (*tr* trace < 0.1. *HUFA* highly unsaturated fatty acids > 20:3). Totals include some minor components not shown. Values within a given row not bearing the same superscript letter are significantly different ($P<0.05$). If no superscript appears, values are not different

Fatty acid	Stage of maturation			
	I	II	III	IV
14:0	0.2±0.0	0.2±0.0	0.2±0.0	0.3±0.0
15:0	0.3±0.0[a]	0.5±0.1[b]	0.5±0.0[b]	0.8±0.0[c]
16:0	4.4±0.5[a]	4.6±0.1[a]	4.6±0.1[a]	7.1±0.2[b]
17:0	0.2±0.1[a]	0.3±0.0[a]	0.6±0.1[b]	1.0±0.1[b]
18:0	3.3±0.3[a]	3.1±0.0[a]	3.3±0.1[a]	5.5±0.1[b]
20:0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
Total saturated	9.4±0.9[a]	9.2±0.2[a]	9.5±0.3[a]	15.0±0.4[b]
16:1 (n-7)	2.3±0.2[a]	2.5±0.1[a]	3.2±0.1[b]	6.5±0.6[c]
18:1 (n-9)	2.7±0.2[a]	2.4±0.1[a]	2.3±0.0[a]	4.2±0.2[b]
18:1 (n-7)	1.6±0.1[a]	1.4±0.0[a]	1.6±0.1[a]	2.9±0.2[b]
20:1 (n-9)	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:1 (n-7)	0.5±0.0	0.4±0.1	0.3±0.1	0.5±0.1
22:1 (n-11)	0.1±0.0	0.1±0.0	tr	0.1±0.0
22:1 (n-9)	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0
Total monoenes	7.6±0.6[a]	7.0±0.0[a]	7.7±0.2[a]	14.4±1.1[b]
16:2	0.5±0.1[a]	0.9±0.0[b]	1.1±0.1[b]	1.3±0.1[b]
16:3	0.9±0.1	0.8±0.1	0.9±0.1	1.4±0.2
16:4	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
18:2 (n-6)	0.8±0.1[a]	0.9±0.1[a]	1.0±0.1[a]	1.5±0.1[b]
18:3 (n-3)	0.4±0.0[a]	0.4±0.0[a]	0.7±0.1	1.5±0.1[c]
18:4 (n-3)	0.3±0.0	0.2±0.1	0.1±0.0	0.3±0.0
20:2 (n-6)	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:3 (n-6)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:3 (n-3)	0.1±0.0	tr	0.1±0.0	0.1±0.0
20:4 (n-6)	3.8±0.4[a]	3.2±0.1[a]	2.2±0.1[b]	2.1±0.1[b]
20:4 (n-3)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:5 (n-3)	4.8±0.5[a]	4.6±0.2[a]	4.6±0.3[a]	5.8±0.1[b]
22:5 (n-6)	0.2±0.0	0.2±0.0	0.2±0.0	0.3±0.0
22:5 (n-3)	0.3±0.0	0.3±0.0	0.4±0.0	0.4±0.0
22:6 (n-3)	1.9±0.2	2.1±0.1	2.1±0.2	2.1±0.1
HUFA (n-6)	4.4±0.5[a]	3.7±0.3[a]	2.8±0.2[b]	3.0±0.2[b]
HUFA (n-3)	7.1±0.7[a]	7.0±0.1[a]	7.2±0.4[a]	8.7±0.3[b]

constant from Stages I to III, then increased significantly by 1.3 to 2 times from Stages III to IV. In contrast, 16:1 (n-7) showed significant increases from Stages II to IV, AA content significantly decreased from Stages II to III and DHA content remained constant throughout maturation.

Variations of the fatty acid contents in total polar lipids from the midgut gland are presented in Table 7. Major fatty acids were 16:0, 18:0, 16:1 (n-7), 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3). The content of these fatty acids was constant from Stages I to II, then decreased significantly (with the exception of 16:0) from Stages II to III (major decreases were presented by AA, EPA and DHA), and then remained constant or increased significantly from Stages III to IV.

The evolution of TAG fatty acid contents of ovaries is indicated in Table 8. The most abundant fatty acids in this fraction were 16:0, 16:1 (n-7), 18:1 (n-9), 18:1 (n-7), 18:2 (n-6), 18:3 (n-3), 20:4 (n-6), 20:5 (n-3) and 22:6 (n-3). A general trend towards significant increases was observed in the concentration of these fatty acids (1.9 to 3.2 times from Stages I to IV). Utmost increments occurred from Stages III to IV.

The variations of TAG fatty acid contents of the midgut gland are given in Table 9. Major fatty acids in this fractions were 14:0, 16:0, 16:1 (n-7), 18:1 (n-9), 18:1 (n-7), 18:3 (n-3) and 20:5 (n-3). A general trend was also observed in the concentration of these fatty acids during maturation: a large significant increase from Stages I to II followed by significant decreases Stages II to III and then significant increases again from Stages III to IV. Unexpectedly, the contents of 18:1 (n-7), 18:2 (n-6) and 18:3 (n-3) showed significant increases from Stages I to IV.

Discussion

Knowledge of the biochemistry and metabolism of the processes involved during sexual maturation are essential for a complete understanding of crustacean reproduction. Maternal nutrition should meet the metabolic costs of biosynthesis and mobilization of nutrients for the manufacture of gonads, oocytes and egg yolk. Thus, the nutritional status of females is likely to be of critical importance for success-

Table 7 *Uca tangeri*. Variations in midgut gland total polar lipid fatty acid contents (μg fatty acid mg^{-1} dry wt) at different stages of sexual maturation. Data are means ± SD ($n = 3$). SD = 0.0 implies an SD of <0.05. (*tr* trace < 0.1. *HUFA* highly unsaturated fatty acids > 20:3). Totals include some minor components not shown. Values within a given row not bearing the same superscript letter are significantly different ($P < 0.05$). If no superscript appears, values are not different

Fatty acid	Stage of maturation			
	I	II	III	IV
14:0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
15:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
16:0	1.9 ± 0.1	2.3 ± 0.4	1.7 ± 0.1	2.3 ± 0.1
17:0	0.2 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
18:0	1.6 ± 0.0^{a}	1.8 ± 0.1^{a}	1.2 ± 0.1^{b}	1.6 ± 0.1^{a}
20:0	0.1 ± 0.0	0.1 ± 0.0	tr	0.1 ± 0.0
Total saturated	4.2 ± 0.1^{a}	4.9 ± 0.3^{a}	3.6 ± 0.1^{b}	4.8 ± 0.2^{a}
16:1 (n-7)	1.2 ± 0.0^{a}	1.4 ± 0.3^{ab}	1.2 ± 0.0^{a}	1.7 ± 0.0^{b}
18:1 (n-9)	0.8 ± 0.0^{a}	1.0 ± 0.1^{a}	0.5 ± 0.0	0.9 ± 0.0^{a}
18:1 (n-7)	0.6 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
20:1 (n-9)	tr	tr	tr	tr
20:1 (n-7)	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
22:1 (n-11)	tr	tr	tr	tr
22:1 (n-9)	tr	tr	tr	tr
Total monoenes	2.9 ± 0.1^{a}	3.5 ± 0.4^{a}	2.3 ± 0.1^{b}	3.4 ± 0.2^{a}
16:2	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
16:3	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.0
16:4	0.4 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.4 ± 0.0
18:2 (n-6)	0.5 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
18:3 (n-3)	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
18:4 (n-3)	tr	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:2 (n-6)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:3 (n-6)	tr	tr	tr	0.1 ± 0.0
20:3 (n-3)	0.1 ± 0.0	0.1 ± 0.0	tr	0.1 ± 0.0
20:4 (n-6)	1.7 ± 0.1^{a}	1.6 ± 0.1^{a}	0.7 ± 0.0^{b}	0.7 ± 0.1^{b}
20:4 (n-3)	tr	tr	tr	tr
20:5 (n-3)	2.2 ± 0.1^{a}	2.5 ± 0.2^{a}	1.4 ± 0.1^{b}	1.7 ± 0.2^{b}
22:5 (n-6)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:5 (n-3)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:6 (n-3)	1.3 ± 0.0^{a}	1.3 ± 0.1^{a}	0.5 ± 0.0^{b}	0.6 ± 0.1^{b}
HUFA (n-6)	2.0 ± 0.1^{a}	1.9 ± 0.1^{a}	1.0 ± 0.0^{b}	1.1 ± 0.2^{b}
HUFA (n-3)	3.6 ± 0.1^{a}	4.1 ± 0.3^{a}	2.1 ± 0.1^{b}	2.7 ± 0.3^{b}

ful maturation and spawning. Lipids play several important roles in the biochemistry, metabolism and reproduction of decapod crustaceans, either as major energy sources or membrane constituents of cells (Harrison 1990).

Increases in total ovarian lipids with maturation has been widely documented in wild and cultured Penaeid females and shrimps (Gehring 1974; Read and Caulton 1980; Galois 1984; Teshima et al. 1988a; Jeckel et al. 1989; Teshima et al. 1989; Harrison 1990; Mourente and Rodríguez 1991), but this type of study on Brachyurans and inter-tidal crabs is scarce in the literature (Lautier and Lagarrigue 1988; Lee and Puppione 1988). The great differences in the life habits of fiddler crabs and of the more extensively studied Decapod Crustaceans (mainly Penaeids) makes the former an interesting subject of investigation. In *Uca tangeri* a significant increase in total lipid content was observed in the ovaries during the final stages of maturation (from Stages III to IV), whereas a significant decrease of midgut gland total lipids was shown in the previous period of maturation, between Stages II and III. We suggest that during this period the depletion of midgut gland total lipids is due to increased metabolic activity (anabolism and catabolism) which is necessary to cover the energetic demand for biosynthesis and mobilization of lipids to the ovary. Moreover, energy demands could also have been partially covered by carbohydrates (from either the ovary or midgut gland), which showed significant decreases from Stages III to IV in both organs.

Phospholipids are considered to be the principal lipid components of the tissues and hemolymph 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). The polar lipid content in the ovary was higher than the neutral lipid in Stage I, but in the succeeding stages, neutral lipids were 1.1, 1.8 and 1.9 times higher than polar lipids. This has also been observed in *Penaeus japonicus* (Teshima et al. 1989). The predominance of the TAG fraction in the ovary and midgut gland indicates that this species has enough food available throughout the maturation period. The increase in total lipids that occurred in the ovaries of *Uca tangeri* during maturation was mainly due to an increase in neutral lipids (primarily TAG), whereas polar lipids (primarily PC and PE) only increased at the end of maturation (from Stages III to

Table 8 *Uca tangeri*. Variations in ovarian triacylglycerol fatty acid contents (μg fatty acid mg^{-1} dry wt) at different stages of sexual maturation. Data are means ± SD ($n=3$). SD = 0.0 implies an SD of 0.05. (*tr* trace < 0.1. *HUFA* highly unsaturated fatty acids > 20:3). Totals include some minor components not shown. Values within a given row not bearing the same superscript letter are significantly different ($P<0.05$). If no superscript appears, values are not different

Fatty acid	Stage of maturation			
	I	II	III	IV
14:0	1.0±0.1 [a]	1.3±0.1 [a]	1.6±0.1 [ab]	2.2±0.1 [b]
15:0	1.7±0.2	1.7±0.2	1.7±0.1	2.2±0.4
16:0	12.1±1.0 [a]	15.0±0.4 [a]	17.5±0.8 [b]	23.8±0.6 [c]
17:0	0.8±0.2 [a]	0.9±0.2 [a]	1.3±0.2 [ab]	3.3±0.6 [b]
18:0	0.4±0.0 [a]	0.8±0.0 [b]	1.4±0.0 [c]	2.8±0.2 [d]
20:0	tr	0.1±0.0	0.1±0.0	0.2±0.0
Total saturated	17.7±1.6 [a]	21.3±0.9 [ab]	25.0±1.2 [b]	34.6±1.7 [c]
16:1 (n-7)	8.2±0.6 [a]	10.6±0.6 [ab]	13.5±0.3 [b]	20.9±1.3 [c]
18:1 (n-9)	4.3±0.6 [a]	4.6±0.1 [a]	4.7±0.1 [a]	6.9±0.2 [b]
18:1 (n-7)	1.6±0.1 [a]	1.9±0.1 [ab]	2.4±0.2 [b]	3.4±0.3 [c]
20:1 (n-9)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:1 (n-7)	0.4±0.1	0.5±0.0	0.5±0.0	0.7±0.1
22:1 (n-11)	tr	tr	0.1±0.0	0.1±0.0
22:1 (n-9)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
Total monoenes	14.7±1.4 [a]	17.9±0.7 [ab]	21.3±0.5 [b]	32.3±1.4 [c]
16:2	0.3±0.0 [a]	0.5±0.0 [a]	0.8±0.1 [b]	1.1±0.1 [b]
16:3	0.2±0.0	0.2±0.0	0.2±0.0	0.3±0.0
16:4	0.2±0.0	0.2±0.0	0.3±0.0	0.4±0.1
18:2 (n-6)	1.2±0.1 [a]	1.7±0.1 [ab]	2.0±0.3 [bc]	2.6±0.1 [c]
18:3 (n-3)	0.6±0.0 [a]	0.9±0.0 [ab]	1.7±0.4 [b]	3.0±0.2 [c]
18:4 (n-3)	0.1±0.0 [a]	0.1±0.0 [a]	0.4±0.0 [b]	0.6±0.1 [b]
20:2 (n-6)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:3 (n-6)	tr	0.1±0.0	0.1±0.0	0.1±0.0
20:3 (n-3)	tr	tr	0.1±0.0	0.2±0.0
20:4 (n-6)	1.2±0.2 [a]	2.0±0.0 [b]	2.5±0.2 [bc]	2.7±0.2 [c]
20:4 (n-3)	0.1±0.0	0.1±0.0	0.2±0.0	0.2±0.0
20:5 (n-3)	3.1±0.4 [a]	4.3±0.3 [b]	6.7±0.5 [c]	8.5±0.5 [d]
22:5 (n-6)	0.2±0.0	0.2±0.0	0.3±0.1	0.4±0.0
22:5 (n-3)	0.1±0.0	0.1±0.0	0.2±0.0	0.3±0.0
22:6 (n-3)	0.6±0.1 [a]	1.0±0.1 [a]	1.7±0.2 [b]	1.9±0.2 [b]
HUFA (n-6)	1.7±0.2 [a]	2.6±0.0 [b]	3.6±0.2 [c]	4.0±0.2 [c]
HUFA (n-3)	4.1±0.6 [a]	5.9±0.4 [a]	9.1±0.7 [b]	11.2±0.8 [b]

IV). Curiously, there was not a concomitant decrease of midgut gland lipids during this period. Depletion of both polar and neutral lipids had occurred previously in the midgut gland, from Stages II to III.

Several studies have shown that the accumulation of oocyte lipids depends on maternal food intake during vitellogenesis and that the midgut gland acts primarily to modify incoming lipids for export to the ovaries (Clarke 1982; Teshima and Kanazawa 1983; Galois 1984; Jeckel et al. 1989; Harrison 1990; Mourente and Rodríguez 1991). Neutral lipid reserves in the midgut gland (mainly TAGs) are converted to polar lipids and exported and transported via the hemolymph to the ovary as high density lipoproteins (HDLs) (Allen 1972; Lee and Puppione 1978; Teshima and Kanazawa 1980; Harrison 1990). PC and PE are the major circulating lipids in the crustacean hemolymph (Gilbert and O'Connor 1970; Allen 1972; Lee and Puppione 1978; Chang and O'Connor 1983; Harrison 1990), whereas ingested neutral lipids are enzymatically cleaved (by esterases and TAG lipases) to either α,β-diacylglycerides or β-monoacylglycerides, both of which are converted to phospholipids by the absorptive cells of the midgut gland and transported to various tissues, including the maturing ovaries, for use as membrane components or converted to reserve lipids (triacylglycerols) during sexual maturation (Chang and O'Connor 1983; Harrison 1990). As to the polar lipid reserves, the amount of total polar lipid fractions is more important than changes in the concentrations of individual polar lipid classes, since their biosynthetic pathways are closely related and interconversion is easily carried out (Chapelle 1986). During maturation, the ovaries become an additional centre for lipid metabolism, including lipogenesis (mainly TAG synthesis). The high levels and significant increases of TAG fraction found in the ovary of *Uca tangeri* seems to denote an active synthesis of this neutral lipid class during maturation. Moreover, the lipids accumulated by the developing oocytes provide the necessary energy for the biosynthetic processes of oogenesis and vitellogenesis (Harrison 1990). It seems evident that all lipid classes (mainly PC, TAG and SE) previously stored in the midgut gland were mobilized and metabolized between Stages II and III (Table 3). However, the extent to which lipids are transferred from the midgut gland or synthesized by the ovaries cannot be de-

Table 9 *Uca tangeri*. Variations in midgut gland triacylglycerol fatty acid contents (μg fatty acid mg^{-1} dry wt) at different stages of sexual maturation. Data are means ± SD ($n=3$). SD = 0.0 implies an SD of <0.05. (*tr* trace < 0.1. *HUFA* highly unsaturated fatty acids >20:3). Totals include some minor components not shown. Values within a given row not bearing the same superscript letter are significantly different ($P<0.05$). If no superscript appears, values are not different

Fatty acid	Stage of maturation			
	I	II	III	IV
14:0	1.3±0.0[a]	4.0±0.2[b]	3.7±0.1[b]	7.4±1.3[c]
15:0	0.9±0.0[a]	3.9±0.2[b]	2.1±0.2[c]	5.5±0.8[d]
16:0	9.0±0.2[a]	21.5±0.5[b]	17.9±0.6[b]	35.4±3.5[c]
17:0	0.5±0.1	0.9±0.3	0.7±0.3	0.9±0.3
18:0	0.6±0.0[a]	1.7±0.0[b]	1.4±0.1[b]	3.8±0.7[c]
20:0	0.1±0.0	0.2±0.0	0.2±0.0	0.3±0.1
Total saturated	13.3±0.3[a]	34.4±0.9[b]	27.9±1.3[b]	52.5±4.8[c]
16:1 (n-7)	7.6±0.2[a]	17.0±0.6[b]	13.1±0.8[c]	24.6±3.7[d]
18:1 (n-9)	1.8±0.1[a]	4.1±0.1[b]	2.6±0.2[c]	7.1±0.9[d]
18:1 (n-7)	1.2±0.0[a]	2.5±0.1[b]	3.0±0.1[c]	5.1±0.3[d]
20:1 (n-9)	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.1
20:1 (n-7)	0.3±0.0[a]	1.1±0.1[b]	0.5±0.1[c]	1.3±0.2[b]
22:1 (n-11)	tr	tr	tr	0.1±0.0
22:1 (n-9)	tr	0.1±0.0	0.1±0.0	0.1±0.0
Total monoenes	11.2±0.3[a]	25.4±0.6[b]	19.7±1.1[bb]	38.9±2.7[d]
16:2	0.2±0.0[a]	0.3±0.0[a]	0.5±0.1[ab]	1.3±0.4[b]
16:3	0.5±0.1[a]	0.5±0.0[a]	0.5±0.1[a]	1.0±0.1[b]
16:4	0.1±0.0[a]	0.6±0.1[b]	0.5±0.1[b]	0.7±0.1[b]
18:2 (n-6)	0.6±0.1[a]	1.4±0.1[b]	1.4±0.1[b]	2.4±0.4[c]
18:3 (n-3)	0.6±0.1[a]	1.8±0.1[b]	2.6±0.2[c]	5.1±0.5[d]
18:4 (n-3)	0.2±0.0[a]	0.8±0.0[bc]	0.5±0.1[b]	1.2±0.2[c]
20:2 (n-6)	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0
20:3 (n-6)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:3 (n-3)	tr	0.1±0.0	0.1±0.0	0.4±0.1
20:4 (n-6)	0.5±0.1[a]	1.2±0.2[b]	0.8±0.1[b]	1.4±0.3[b]
20:4 (n-3)	0.1±0.0	0.2±0.0	0.1±0.0	0.3±0.1
20:5 (n-3)	1.4±0.1[a]	4.6±0.2[b]	3.0±0.2[c]	6.7±1.8d
22:5 (n-6)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
22:5 (n-3)	0.1±0.0	0.2±0.0	0.1±0.0	0.2±0.1
22:6 (n-3)	0.4±0.0[a]	0.9±0.2[b]	0.4±0.1[a]	1.0±0.2[b]
HUFA (n-6)	0.9±0.1[a]	2.2±0.1[bc]	1.7±0.1[b]	2.5±0.3[c]
HUFA (n-3)	2.1±0.1[a]	6.4±0.2	4.0±0.3[c]	9.2±2.3[b]

duced from these data. Nevertheless, life cycle and energetic strategy may differ significantly from species to species.

Cholesterol is an essential nutrient for crustaceans because they are incapable of synthesizing de novo the steroid ring (Teshima and Kanazawa 1971; Teshima 1982). Cholesterol, in addition to its role as a membrane constituent, was found to be a precursor of steroid hormones, and its presence in the oocyte plays an essential role during embryogenesis and larvae development (Kanazawa and Teshima 1971; Blanchet-Tournier 1982). No variations were observed in cholesterol levels either in the ovary or the midgut gland of *Uca tangeri* during development. Thus, dietary cholesterol accumulated in the ovaries will be used by the larvae for their development, since larvae of *Penaeus japonicus* were also found unable to synthesize cholesterol from acetate (Teshima et al. 1983). Furthermore, the ovary is presumably the major site of cholesterol metabolism followed by the midgut gland (Kanazawa et al. 1988).

Several compositional and nutritional studies have postulated that (n-3) and (n-6) HUFA (highly unsaturated fatty acids) are involved in some capacity in the reproductive processes of crustaceans (Harrison 1990; Mourente et al. 1990; Alava et al. 1993). Furthermore, EPA and DHA have been shown to be major components of phospholipids belonging to the eye membranes of the shrimp *Pandalus borealis* (Bell and Dick 1990), indicating that these fatty acids may play an important role in visual and neural tissues of marine crustaceans. In the present study, ovarian lipids contained higher proportions of AA, EPA and DHA than the midgut gland (Tables 4 and 5). The AA level remained constant in ovarian total lipids during maturation, but decreased significantly in total polar lipids, whereas an upward trend was shown in the TAG fraction (Tables 6 and 8). AA is an important precursor of prostaglandins, and it is likely that part of (n-3) and (n-6) PUFA may also be used as precursors for these compounds. Prostaglandins, synthesized from specific PUFA of membrane-bound phospholipids, have important physiological implications which include regulation of ion flux, temperature regulation and reproductive biology (oocyte maturation, egg production or hatching control) (Holland et al. 1985; Stanley-Samuelson 1987).

EPA and DHA contents in total lipids of the ovary increased significantly from Stages II to IV and II to III, respectively, whereas in midgut gland total lipids a concomitant decrease of these fatty acids was observed. No significant changes were shown by 20:5 (n-3) and 22:6 (n-3) in ovarian polar lipids during maturation with the exception of a significant increase presented by the former from Stages III to IV. In contrast, both fatty acids showed upward trends during the same period in the TAG fraction in the same organ. This may indicate an accumulation of these particular fatty acids in oocytes in order to be used subsequently during embryogenesis and early larval development. A limited ability for biosynthesis of 20:5 (n-3) and 22:6 (n-3) from 18:3 (n-3) has been observed in juvenile and adult crustaceans (Kanazawa et al. 1979; Teshima et al. 1992 a).

However, in larvae of *Penaeus japonicus* bioconversion processes occurred to a greater extent, indicating a more active turnover of 18:3 (n-3) and suggesting a subsequent variation in fatty acid metabolism during metamorphosis (Teshima et al. 1992 b). In contrast, there is a lack of information about the capacity of fatty acid biosynthesis (synthesis de novo, desaturation and elongation) in organs such as the ovary or midgut gland during maturation, which is of great interest since mobilized fatty acids of dietary origin and de novo synthesized ones will accumulate in the ovaries as energy and membrane constituent reserves. Radioisotope experiments suggest that dietary essential PUFAs are selectively sequestered within the oocyte as a component of yolk polar lipids. Thus, they can satisfy essential fatty acid requirements during oogenesis, embryogenesis and non-feeding larval stages, while non-essential fatty acids become a component of the oocyte TAG stores to be used as fuel by the embryos and pre-feeding larvae (Teshima et al. 1988 b). Further radiotracer, radioimmunoassays and enzyme research is needed to advance in our knowledge on nutrient storage, mobilization, valorization of nutrients and transfer of energy to the gonads during maturation.

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