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SEASONAL VARIATIONS IN THE FATTY ACIDS OF THE TRIACYLGLYCEROLS AND PHOSPHOLIPIDS OF TWO POPULATIONS OF ADULT CLAM (*TAPES DECUSSATUS* L. AND *T. PHILIPPINARUM*) REARED IN A COMMON HABITAT

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Abstract—1. Seasonal variations in fatty acid composition of the triacylglycerols and phospholipids of an indigenous and an introduced clam species reared in a common habitat were analyzed using gas–liquid capillary column chromatography.

2. Despite different geographical origins, the fatty acid spectra of the two related species were quite similar.

3. In both species the fatty acids of the triacylglycerol fraction, but not the phospholipid fraction, showed clear seasonal variation corresponding to the annual temperature cycle and not to reproductive activity.

4. It is argued that such variations are related to the diet and are not endogenous.

INTRODUCTION

The fatty acids of marine organisms have been the subject of considerable study, notably since the introduction of capillary-column gas chromatography (for review see Ackman, 1980). Although the interpretation of the physiological significance of fatty acid spectra is obviously more meaningful when the spectrum of each major lipid class is examined, such studies have rarely been performed in bivalve molluscs. In those studies where lipid separation has been carried out before fatty acid analysis, the fractionation is often insufficient to provide clear comparisons between triacylglycerols and phospholipids (Ueda, 1974). Where distinct separations have been performed the seasonal variation of the component fatty acids has not been studied (Rodegker and Nevenzel, 1964; Gardner and Riley, 1972; Watanabe and Ackman, 1974).

The present work compares the seasonal variations in the triacylglycerol and phospholipid component fatty acids of an indigenous North Atlantic clam species, *Tapes decussatus* L. (Jeffreys, 1863), and an introduced Indo-Pacific species, *T. philippinarum* (Adams and Reeve, 1850), reared in a common habitat in the Sud-Finistère region of the French Atlantic coast.

MATERIALS AND METHODS

The site and characteristics of each population have been described by Beninger and Lucas (1984), and the lipid extraction and separation techniques have been detailed previously (Beninger, 1984). Two lipid extractions from pooled clam tissue were separated into triacylglycerol and phospholipid fractions at each monthly sampling, and methylation and analysis performed on each fraction. This provided a constant indication of repeatability of analyses between samples from each pool.

Methylation of the component fatty acids was performed using a modified version of the rapid, economical method used by Christopherson and Glass (1969), Vigneron *et al.* (1973) and Prevot and Mordret (1976). One millilitre of reagent-grade hexane was added to 5–40 mg of lipid (triacylglycerol or phospholipid) in a small test-tube and the solution/suspension was mixed for 10 sec using an electric agitator. Two-tenths of a millilitre of 2.7 M NaOH was added, and the stoppered tube was again agitated for 10 sec and heated in a hot-water bath at 50°C for 1 min before being agitated again for 10 sec. Four-tenths of a millilitre of the supernatant was transferred to a weighed vial, evaporated under nitrogen and the fatty acid methyl esters dissolved in 0.17 ml of hexane for each mg of ester. The resulting solution was stored under nitrogen at −80°C in hermetically sealed vials until gas chromatographic analysis was performed.

In order to compare the fatty acid spectra of lipids methylated using this rapid method with those methylated using a standard KOH–methanol–HCl method (Stephan, 1976), a reference oil (cod-liver oil) was methylated using both techniques. The resulting fatty acid spectra were found to be identical. The same comparison was then made on *T. philippinarum* lipids of May and June 1980, and the resulting spectra were also found to be identical. The calculated distance values of 1.71–2.99 were well within the normal range of variation for two subsamples analysed using identical techniques (Bottino *et al.*, 1980).

The fatty acid methyl esters were separated using a Packard model 420 capillary-column gas chromatograph equipped with a Ross injector, an ICAP5 integrator and a Servotrace recorder. The FFAP glass capillary column dimensions were 3.0 m × 0.35 mm i.d. with a helium carrier gas at a relative pressure of 4 kPa. The injector and oven

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temperatures were 220 and 200°C respectively. Fatty acids were identified according to equivalent chain length using a cod-liver-oil reference standard. The capillary column was standardized 12 times at regular intervals during the 2-month analysis period. Certain acids were identified using Potteau's (1974) calculation, while others were either wholly or partially identified following hydrogenation (Farquhar *et al.*, 1959).

Gas-chromatographic analyses were performed on the monthly samples from April 1979 to March 1980 for each species, with additional analyses in May and June 1980 for *T. philippinarum*, as mentioned previously. Distance indices were calculated between species for each month and each fraction (see Bottino *et al.*, 1980).

Reproductive activity was assessed using two indices described previously (Beninger and Lucas, 1984). The first index was the temporal variation of the ash-free dry weight (AFDW) of a standard animal, which should normally reveal the onset of accumulation of organic matter for reproduction. The second index was the presence or absence of oocytes in the female gonad, which allows a precise evaluation of the duration and termination of spawning.

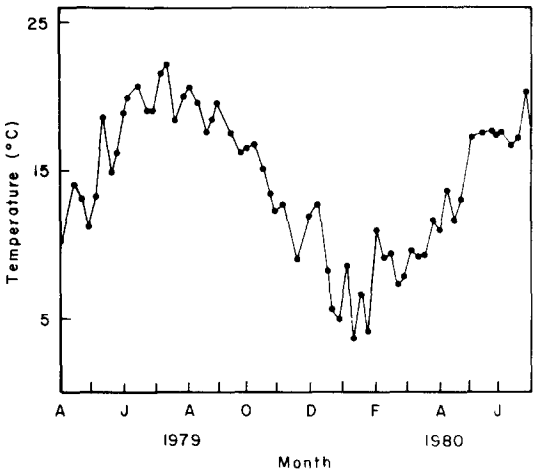

Fig. 1. Temperature cycle in Kermor rearing pond. April 1979–August 1980.

RESULTS

The temperature data for the sampling period showed a pronounced seasonal cycle (Fig. 1). Detailed results of the fatty acid analyses are presented in Tables 1–4. Excellent reliability was achieved in the paired analyses of each component (triacylglycerol or phospholipid) and so only one set of data is presented here. Technical difficulties resulted in non-hermetic seals in certain samples; data from these samples were not included in the tables. The samples affected were the triacylglycerol fatty acids of February 1980 (*T. decussatus*) and April 1980 (*T. philippinarum*) and the phospholipids of November 1979 and February 1980 (*T. decussatus*). Figures 2 and 3 summarize the results for each major fatty acid family.

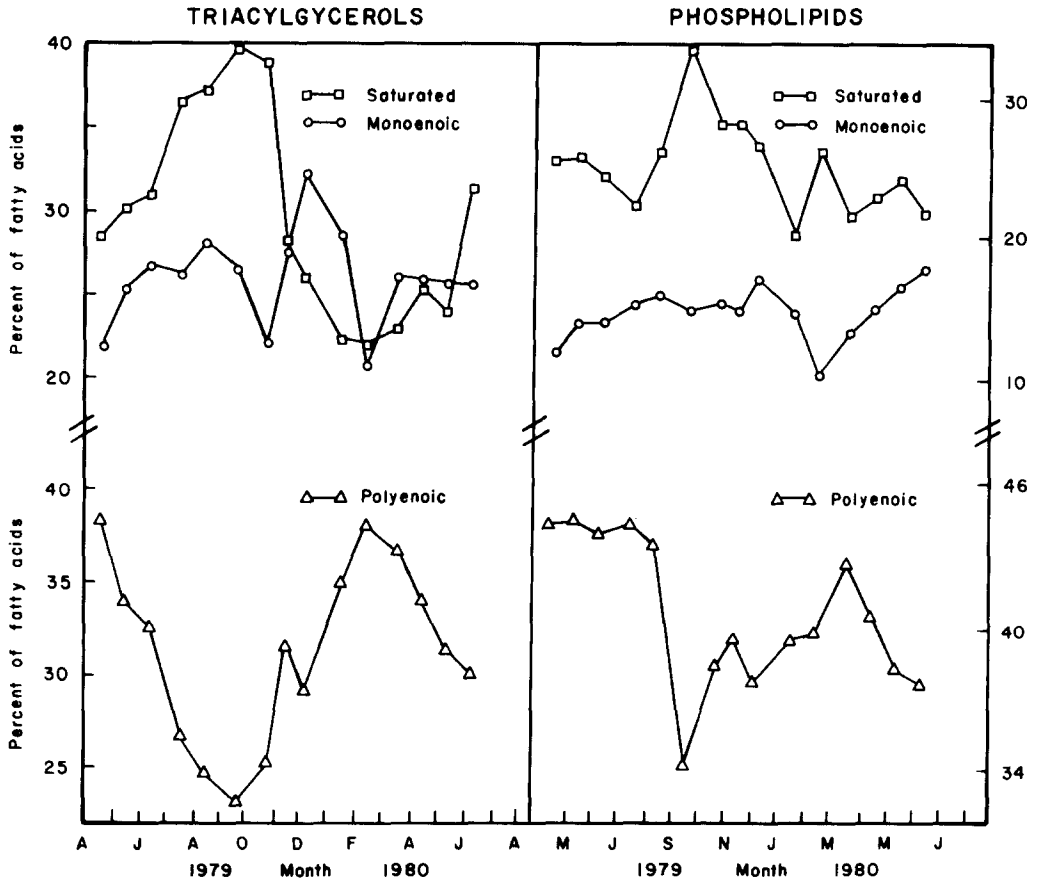

Fig. 2. Variations in the levels of total saturated, monoenoic and polyenoic fatty acids of the triacylglycerols and phospholipids of *T. philippinarum*, April 1979–June 1980.

Table 1. Fatty acid composition of the triacylglycerols of *T. philippinarum*. April 1979–June 1980

FATTY ACID	1979									1980				
	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN.	FEB.	MARCH	MAY	JUNE
SATURATED														
14 : 0	3.95	5.25	5.92	5.75	4.68	4.29	4.09	2.25	2.23	1.34	1.61	1.28	2.22	2.96
15 : 0	0.63	0.62	0.65	1.01	0.96	0.97	1.18	1.00	1.04	0.78	0.71	0.77	0.83	0.84
16 : 0	19.94	19.40	19.18	23.77	24.84	27.05	26.26	17.90	16.66	13.63	13.45	14.83	17.11	21.50
17 : 0	0.69	0.61	0.65	0.87	0.96	1.10	1.10	0.91	0.90	0.96	0.77	0.97	0.83	0.84
18 : 0	3.13	3.89	4.08	4.58	5.08	5.85	5.97	6.04	5.20	5.43	5.50	5.10	4.85	4.70
19 : 0	0.21	0.17	0.23	0.25	0.24	—	—	—	—	—	—	—	—	—
20 : 0	—	0.24	0.26	0.36	0.40	0.43	0.22	—	—	0.19	—	—	—	—
Σ	28.55	30.30	31.00	36.60	37.20	39.69	38.82	28.10	26.03	22.33	22.04	22.95	25.84	30.84
MONOENOIC ω7														
16 : 1	9.16	11.42	12.41	10.94	10.28	9.42	9.42	9.89	12.34	9.69	9.55	8.25	9.26	8.50
18 : 1	5.64	6.49	7.66	7.14	7.14	5.71	5.66	5.50	6.48	4.47	3.97	5.14	6.85	7.00
20 : 1	1.15	1.44	1.42	1.46	1.44	1.56	1.65	1.97	2.10	2.29	2.06	2.39	1.93	1.38
Σ	15.95	19.35	21.49	19.54	18.86	16.69	16.73	17.36	20.92	16.45	15.58	15.78	18.04	16.88
ω9														
16 : 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18 : 1	3.17	3.11	2.24	3.16	4.40	5.21	4.83	4.64	5.35	6.23	4.56	5.20	4.32	4.07
20 : 1	1.04	2.90*	1.17	3.56*	4.48*	0.43	0.50*	0.65	0.94	1.02	0.63*	0.80	1.72	1.38
Σ	4.21	6.01	3.41	6.72	9.28	5.64	5.33	5.29	6.29	7.25	5.19	6.00	6.04	5.45
POLYENOIC ω6														
18 : 2	1.44	1.09	0.98	0.94	1.01	0.91	0.87	0.68	0.58	0.55	0.47	0.74	1.05	1.08
18 : 3	0.86	0.85	0.99	0.83	0.55	0.18	0.23	0.35	0.36	0.12	0.19	0.14	0.40	0.35
20 : 2	2.00	1.63	1.54	1.43	1.60	1.70	1.77	2.12	2.07	2.48	1.88	2.53	2.24	1.96
20 : 3	0.25	0.29	0.21	0.22	0.16	0.24	0.23	0.44	0.53	0.56	0.77	0.41	0.26	0.25
20 : 4	0.80	0.80	0.79	0.90	0.90	1.06	1.27	1.42	1.25	1.67	1.54	2.12	1.45	1.22
22 : 4	0.15	0.11	—	0.12	0.10	0.24	0.19	0.25	0.28	0.33	0.29	0.36	0.23	0.21
22 : 5	0.13	0.11	—	0.14	0.14	0.15	0.18	0.25	0.25	0.28	0.30	0.27	0.23	0.21
Σ	5.63	4.77	4.51	4.58	4.46	4.48	4.74	5.51	5.32	5.99	5.44	6.57	5.86	5.28
ω3														
18 : 3	3.22	2.25	1.57	1.76	2.43	2.59	2.34	2.03	1.91	2.20	1.75	2.19	2.63	3.25
18 : 4	4.77	3.01	2.51	2.28	2.52	3.07	2.51	2.00	1.94	1.69	1.67	2.10	2.67	3.56
20 : 3	1.57	0.80	0.60	0.60	0.71	0.74	0.79	0.77	0.78	1.08	0.81	0.90	0.86	0.86
20 : 4	1.75	1.41	1.32	1.17	1.08	1.01	1.09	1.17	1.21	1.49	1.39	1.37	1.35	1.30
20 : 5	12.66	15.80	16.70	11.08	8.35	6.05	7.91	11.36	9.97	11.56	14.85	12.71	10.90	10.20
22 : 5	1.59	1.09	0.98	1.08	0.99	0.87	1.04	1.47	1.42	1.64	2.32	1.79	1.21	0.94
22 : 6	7.11	4.67	4.41	4.09	4.16	4.32	4.78	7.27	6.62	9.28	9.78	9.04	6.10	5.60
Σ	32.67	29.03	28.09	22.06	20.22	18.65	20.46	26.07	23.85	28.94	32.57	30.10	25.72	25.71
UNIDENTIFIED	8.02	5.63	8.24	6.06	5.67	10.44	6.34	13.47	13.29	13.89	8.20	12.95	12.99	10.95

*Two acids included in one peak.

Table 2. Fatty acid composition of the triacylglycerols of *T. decussatus*, April 1979 June 1980

FATTY ACID	1979									1980	
	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN.	MARCH
SATURATED											
14 : 0	4.17	7.57	10.43	6.17	5.78	4.20	1.92	3.96	1.16	1.48	2.06
15 : 0	0.55	0.65	0.65	0.92	0.85	0.74	0.78	0.71	0.52	0.63	0.66
16 : 0	15.49	17.71	18.48	22.68	22.37	18.03	19.00	16.92	12.50	14.70	16.45
17 : 0	0.80	0.54	0.42	0.83	0.79	0.92	1.08	1.11	0.52	1.24	1.01
18 : 0	4.28	4.10	4.07	4.61	5.23	6.33	7.43	7.50	6.28	7.60	6.32
19 : 0	0.20	0.23	0.26	0.21	0.20	0.19	-	-	-	-	-
20 : 0	-	-	-	0.21	0.21	0.12	-	-	-	-	-
Σ	25.50	30.80	34.30	35.60	35.40	30.53	30.21	30.20	20.98	25.65	26.60
MONOENOIC											
ω7											
16 : 1	8.45	15.43	18.43	10.99	14.17	8.36	8.74	8.20	5.61	5.90	9.08
18 : 1	7.22	6.92	6.93	8.77	8.36	8.60	6.18	5.31	3.70	5.70	5.80
20 : 1	1.39*	0.97*	1.01*	1.14*	1.14*	0.87*	1.25	1.27*	0.97	1.78	1.58
Σ	17.06	23.32	26.37	20.90	23.67	17.83	16.17	14.78	10.28	13.38	16.46
ω9											
16 : 1	-	-	-	-	-	-	-	-	-	-	-
18 : 1	2.46	3.90	3.44	4.13	5.33	5.35	5.14	4.59	4.90	4.40	4.80
20 : 1	0.89	2.08	1.01	3.07	3.71	4.14	0.34	0.27	0.18	0.40	0.30
Σ	3.35	5.98	4.45	7.20	9.04	9.49	5.48	4.86	5.08	4.80	5.10
POLYENOIC											
ω6											
18 : 2	1.30	1.09	0.75	1.14	0.86	1.41	0.72	0.57	0.70	0.62	0.65
18 : 3	1.05	1.00	1.16	1.32	0.68	0.51	0.07	-	-	-	-
20 : 2	1.58	0.97	0.85	1.07	1.06	1.52	1.50	1.43	1.31	1.90	1.70
20 : 3	0.19	0.16	0.85	0.14	0.17	0.15	0.16	1.33	2.14	-	0.52
20 : 4	1.52	1.02	0.82	1.12	1.36	1.72	1.89	1.44	1.53	2.94	2.17
22 : 4	0.21	0.19	0.11	0.12	0.13	0.30	0.33	0.35	0.44	0.72	0.43
22 : 5	0.12	0.14	0.11	0.15	0.17	0.36	0.29	0.26	0.32	0.43	0.39
Σ	5.91	4.57	4.65	5.06	4.43	5.97	4.96	5.38	6.44	6.61	5.86
ω3											
18 : 3	1.77	1.12	0.51	1.57	1.17	1.98	1.38	1.15	0.65	0.82	1.38
18 : 4	2.87	1.91	1.38	1.53	1.45	1.97	1.73	1.48	1.37	0.44	1.80
20 : 3	1.56	0.95	0.14	0.22	0.21	0.35	0.39	0.24	0.71	0.35	0.46
20 : 4	1.00	0.58	0.63	0.47	0.52	0.53	0.58	0.49	0.73	0.63	0.66
20 : 5	13.77	11.96	11.42	5.75	6.23	7.39	7.54	6.95	5.85	8.75	9.41
22 : 5	1.80	1.08	0.81	0.78	1.20	1.34	1.34	1.35	1.24	2.09	1.65
22 : 6	6.72	3.34	2.01	2.90	3.92	7.79	6.34	5.26	6.22	8.79	7.30
Σ	29.49	20.94	16.90	13.22	14.70	21.35	19.30	16.92	16.77	21.87	22.66
UNIDENTIFIED	9.21	8.05	7.18	7.89	8.40	9.81	17.51	11.98	22.28	19.52	16.60

*Two acids included in one peak.

Table 3. Fatty acid composition of the phospholipids of *T. philippinarum*, April 1979–March 1980

FATTY ACID	1979									1980				
	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN.	FEB.	MARCH	MAY	JUNE
SATURATED														
14 : 0	1.78	2.28	1.80	0.87	1.13	0.42	0.44	0.31	0.51	0.29	0.20	0.34	1.11	1.14
15 : 0	0.35	0.33	0.33	0.29	0.38	0.53	0.62	0.51	0.60	0.44	0.43	0.42	0.42	0.48
16 : 0	14.36	14.87	13.89	12.99	15.14	19.60	15.48	14.90	13.52	10.28	13.28	11.86	14.90	14.63
17 : 0	1.06	1.03	0.91	1.17	1.32	2.15	1.80	1.91	1.76	1.37	1.83	1.34	1.40	1.43
18 : 0	7.51	7.23	7.47	7.01	8.12	10.85	9.88	10.52	10.24	7.85	10.47	7.73	7.92	7.65
19 : 0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20 : 0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Σ	25.56	25.74	24.40	22.33	26.09	33.55	28.22	28.15	26.63	20.23	26.30	21.69	25.75	25.33
MONOENOIC														
ω7														
16 : 1	2.52	3.76	3.82	2.91	2.50	2.14	3.02	3.00	3.90	3.99	2.38	3.14	2.68	3.00
18 : 1	2.41	2.74	2.01	2.61	4.04	1.58	1.52	1.76	2.20	1.39	1.38	1.46	3.12	2.87
20 : 1	1.58	1.71	1.74	1.80	1.92	2.01	1.90	1.90	2.16	1.97	2.10	1.90	1.82	2.00
Σ	6.51	8.21	7.57	7.30	8.46	5.73	6.44	6.66	8.26	7.35	5.86	6.50	7.62	7.87
ω9														
16 : 1	—	—	—	1.62	—	—	—	—	—	—	—	—	0.75	1.04
18 : 1	1.97	2.09	2.62	2.33	2.50	4.31	4.51	4.30	4.35	4.04	3.64	3.80	2.86	3.02
20 : 1	1.32	1.71	3.77*	3.94*	4.97*	0.62	0.55	0.71	0.85	0.83	0.81*	0.80	2.53	2.05
Σ	3.29	3.80	6.39	7.95	7.47	4.93	5.06	5.01	5.20	4.87	4.45	4.60	6.14	6.24
POLYENOIC														
ω6														
18 : 2	0.41	0.48	0.31	0.69	0.41	0.46	0.38	0.35	0.28	—	—	0.30	0.56	0.56
18 : 3	0.37	0.47	0.43	0.26	0.33	—	—	—	—	—	—	—	—	0.12
20 : 2	1.70	1.58	1.53	1.44	1.71	1.68	1.69	1.34	1.50	1.15	1.34	1.28	1.53	1.53
20 : 3	0.16	0.19	0.23	0.18	0.18	—	—	0.20	—	—	—	0.22	0.31	0.21
20 : 4	2.22	1.77	1.96	2.27	2.79	2.48	3.23	3.20	3.56	3.85	3.70	3.28	2.53	3.00
22 : 4	0.98	0.70	0.73	—	0.89	0.95	1.20	1.22	1.51	1.63	1.65	1.52	1.05	1.11
22 : 5	0.68	0.46	0.63	—	0.44	0.64	0.80	0.84	0.90	1.03	0.97	0.87	0.65	0.82
Σ	6.52	5.65	5.82	4.84	6.75	4.70	7.30	7.05	7.75	7.66	7.66	7.47	6.63	7.35
ω3														
18 : 3	2.20	2.48	1.89	1.93	3.07	3.59	3.21	2.86	2.38	1.22	2.08	1.70	1.69	1.40
18 : 4	1.91	1.42	1.13	1.34	1.45	1.14	1.21	1.10	0.84	0.80	0.43	1.31	1.21	1.27
20 : 3	0.54	0.29	0.20	0.26	2.21	0.26	0.21	0.20	0.24	0.14	0.12	0.14	0.29	0.26
20 : 4	1.35	1.29	1.14	0.93	1.10	0.35	0.43	0.42	0.67	0.44	0.34	0.64	1.13	0.74
20 : 5	11.87	16.60	17.31	15.46	12.67	8.80	9.61	10.64	9.56	9.09	9.44	10.52	8.91	10.30
22 : 5	3.93	3.40	3.43	4.03	3.63	2.96	3.31	3.64	3.55	4.11	4.17	4.20	3.89	2.84
22 : 6	16.10	13.40	13.08	15.56	14.69	12.46	13.19	13.67	12.81	16.08	15.68	16.70	11.80	13.90
Σ	37.87	38.88	38.18	39.54	36.82	29.56	31.20	35.53	30.05	31.88	32.26	35.35	28.92	30.71
UNIDENTIFIED	14.00	11.13	11.51	12.73	8.68	15.02	15.51	14.74	17.21	15.52	17.74	16.95	15.87	16.91

*Two acids included in one peak.

Table 4. Fatty acid composition of the phospholipids of *T. decussatus*. April 1979 March 1980

FATTY ACID	1979								1980	
	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	DEC.	JAN.	MARCH
SATURATED										
14 : 0	1.58	2.49	3.08	2.12	1.43	2.95	0.60	0.45	0.36	0.55
15 : 0	0.32	0.34	0.40	0.43	0.37	0.50	0.46	0.43	0.35	0.41
16 : 0	9.97	9.88	13.95	13.73	9.73	14.81	11.02	9.73	8.60	11.33
17 : 0	1.10	1.10	1.27	1.53	1.42	2.09	1.91	2.05	1.83	2.09
18 : 0	8.29	8.20	10.38	9.60	8.24	12.15	10.06	11.02	9.45	10.62
19 : 0	-	-	-	0.12	0.21	-	-	-	-	-
20 : 0	-	-	-	-	-	-	-	-	-	-
Σ	21.26	22.01	29.08	27.53	21.40	32.50	24.05	23.68	20.59	25.00
MONOENOIC										
ω7										
16 : 1	3.47	4.57	5.76	3.19	3.30	3.09	2.28	2.49	2.61	2.86
18 : 1	2.50	2.52	2.83	2.11	2.63	3.61	1.30	1.24	1.28	1.75
20 : 1	1.50	1.80	2.00	2.11	2.11	1.75	1.79	1.73	1.64	1.98
Σ	7.47	8.89	10.59	7.41	8.04	8.45	5.37	5.46	5.53	6.59
ω9										
16 : 1	0.82	0.75	-	-	-	-	-	-	-	-
18 : 1	2.70	2.57	2.97	3.84	3.43	5.81	4.59	4.59	4.05	4.49
20 : 1	1.20	0.85	0.91	3.84*	3.43*	1.46*	1.31	1.36	0.60	1.23
Σ	4.72	4.17	3.88	7.68	6.86	7.27	5.90	5.95	4.65	5.72
POLYENOIC										
ω6										
18 : 2	0.40	0.39	0.25	0.56	0.39	0.63	-	0.15	0.20	-
18 : 3	0.17	0.27	0.16	0.34	0.40	0.30	-	-	-	-
20 : 2	1.15	1.05	1.07	1.68	1.26	1.41	1.32	1.00	0.81	0.96
20 : 3	0.24	0.24	0.27	0.20	0.30	-	-	-	0.13	0.13
20 : 4	3.47	3.08	2.47	2.72	3.72	3.02	4.06	4.49	5.10	4.03
22 : 4	1.38	1.47	1.11	1.18	1.54	1.40	1.90	2.03	2.40	1.97
22 : 5	0.84	0.76	0.60	0.76	0.88	0.69	1.03	0.99	1.17	0.97
Σ	7.65	7.26	5.93	7.44	8.49	7.45	8.31	8.66	9.61	8.06
ω3										
18 : 3	2.41	2.12	3.57	4.35	2.34	4.04	3.10	2.87	2.40	2.82
18 : 4	1.20	1.06	0.68	0.95	0.71	0.63	0.33	0.61	0.45	1.05
20 : 3	0.23	0.13	-	-	-	-	-	-	-	-
20 : 4	0.73	0.57	0.58	0.39	0.50	0.47	-	0.12	0.14	0.21
20 : 5	10.03	10.91	11.31	7.43	8.55	4.70	5.95	5.54	6.74	6.47
22 : 5	5.15	4.74	3.68	4.12	4.71	3.21	4.40	4.32	5.25	4.06
22 : 6	14.23	12.47	8.00	10.33	12.47	10.06	14.11	13.69	15.70	13.11
Σ	33.98	32.00	27.82	27.57	29.28	23.11	27.89	27.15	30.68	27.72
UNIDENTIFIED	19.22	18.80	15.16	14.30	17.99	15.62	23.95	22.77	22.55	21.18

*Two acids included in one peak.

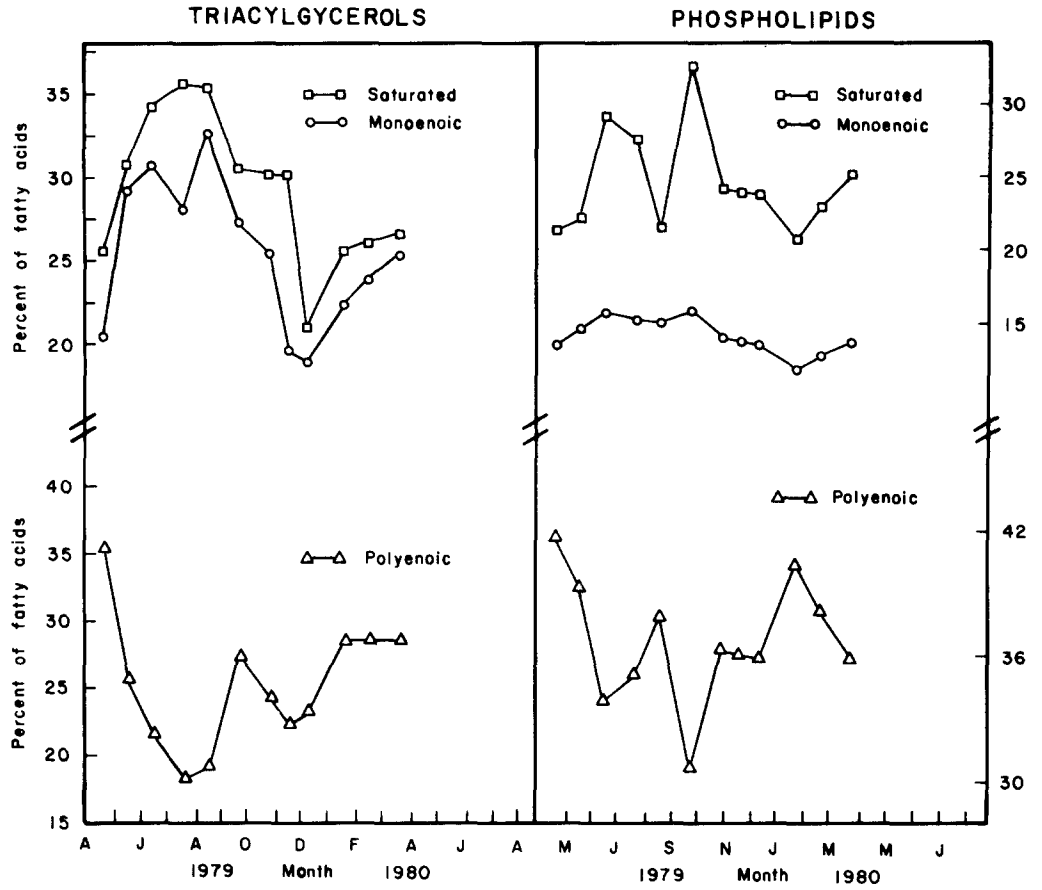

Fig. 3. Variations in the levels of total saturated, monoenoic and polyenoic fatty acids of the triacylglycerols and phospholipids of *T. decussatus*, April 1979–March 1980.

Triacylglycerols

Saturated acids. The dominant triacylglycerol fatty acids in decreasing order of importance were C16:0, C14:0 and C18:0 (Tables 1 and 2).

The seasonal variation in the triacylglycerol total saturated acids was quite similar in both species (Figs 2 and 3). In both cases an increase from spring to late summer 1979 was followed by a decrease during the autumn and winter. A progressive increase occurred once again from mid- (*T. decussatus*) or late winter (*T. philippinarum*) to the end of the sampling periods in spring and summer 1980.

The C14:0 and C16:0 acids were more abundant in the triacylglycerols than in the phospholipids in both species (Tables 1–4). *Tapes decussatus* triacylglycerols contained greater levels of C14:0, while *T. philippinarum* triacylglycerols contained greater levels of C16:0.

Monoenoic acids. The major monoenoic fatty acids in the triacylglycerols of these two species were, in order of decreasing importance: C16:1 ω7, C18:1 ω7 and C18:1 ω9.

The variations of the triacylglycerol monoenoic acids were similar to those of the saturated acids in *T. decussatus* but less so in *T. philippinarum* (Figs 2 and 3). The difference is mainly due to the increased level of C16:1 ω7 in *T. philippinarum* in November and December 1979.

Among the triacylglycerols the levels of the ω7 fatty acids were always 2–6 times greater than those of the ω9 fatty acids (Tables 1 and 2). Apart from the C16:1 ω7, there was virtually no difference in seasonal monoenoic fatty acid variations in the triacylglycerols of these two species.

Polyenoic acids. The seasonal variations in the sum of the triacylglycerol polyenoic acids were quite similar in both species (Figs 2 and 3). A rapid decrease from spring to late summer was followed by an increase in autumn, a partial decrease in early winter and a subsequent increase and stabilization in *T. decussatus* for the winter and early spring. In *T. philippinarum* the additional summer samples showed a decrease in spring and summer 1980 similar to that observed in the previous year.

The sum of the ω3 acids was always 4–6 times greater than that of the ω6 acids (Tables 1 and 2). The amounts of individual ω6 acids never exceeded 3%, while some ω3 acids (especially C20:5 ω3) varied from 6 to 17%. In both species the C18:2 ω6 and C18:3 ω6 levels were more abundant among the triacylglycerols than the phospholipids (Tables 1–4).

Phospholipids

Saturated acids. In decreasing order of importance, the C16:0 and C18:0 were the dominant saturated fatty acids of the phospholipids (Tables 3 and 4). In

T. decussatus these two acids were present in equal amounts, while in *T. philippinarum* the levels of C16:0 were twice as great as those of C18:0 for most of the year. The C18:0 level was greater in the phospholipids than in the triacylglycerols. In contrast to the triacylglycerols, the seasonal variation in the sum of the saturated acids was very irregular in the phospholipid fraction (Figs 1 and 2).

Monoenoic acids. The individual monoenoic acids were all present at levels of less than 6% (Tables 3 and 4). No clear seasonal variation was evident in the individual acids of this group; however, the variation in the sum of the phospholipid monoenoic acids showed the same relationships to the saturated acids as was previously observed in the triacylglycerols of each species. In *T. decussatus* the total monoenoic acid variations followed those of the saturated acids quite closely, while this was not true of the *T. philippinarum* acids (Figs 2 and 3).

Polyenoic acids. Among the individual phospholipid polyenoic acids, *T. decussatus* contained greater levels of C18:3 ω3, C20:4 ω6 and C22:4 ω6, while *T. philippinarum* showed greater levels of C20:5 ω3, C20:4 ω3 and C20:3 ω3 (Tables 3 and 4). The phospholipids of both species contained greater polyenoic acid levels than the triacylglycerols, especially C18:3 ω3, C22:4 ω6, C22:5 ω3, C22:5 ω6 and C22:6 ω3.

The sum of the phospholipid polyenoic acids decreased rapidly in spring and summer for *T. philippinarum* (Figs 2 and 3). The variations in the sum of these acids were similar in both species from August 1979 to the end of the sampling period, with decreases between August and September 1979, progressive increases until late winter and early spring, and finally a marked progressive decrease.

Distances between the fatty acid spectra of the two species

The distances between the fatty acid spectra of the triacylglycerols and phospholipids of each species are presented for each month of the sampling period (Table 5). The values range between 4.8 and 11.1 for the triacylglycerols and between 4.3 and 10.7 for the phospholipids. No clear pattern is shown in the distance values of the triacylglycerol spectra as compared to the phospholipid spectra.

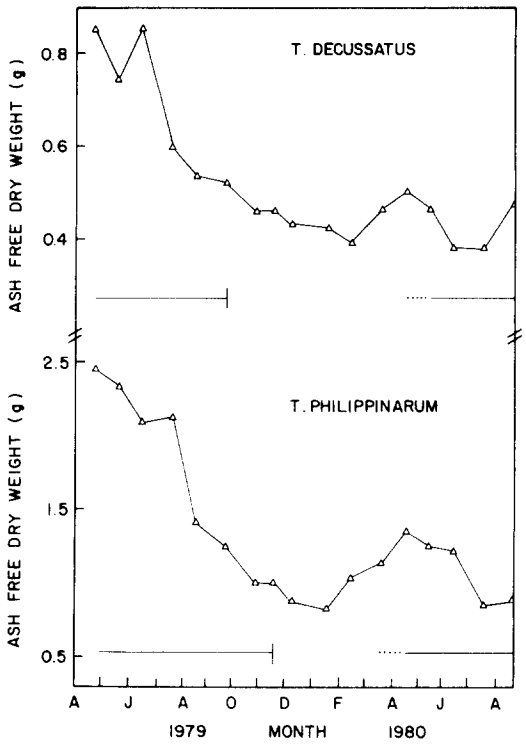

Fig. 4. Indices of reproductive activity of *T. decussatus* and *T. phillippinarum*. The curve represents the ash-free dry weight (AFDW) of the standard animal, while the horizontal bar represents the period during which mature oocytes were observed in the gonad. The dotted line indicates the period during which immature oocytes were observed in the gonad.

Unidentified fatty acids

Unidentified acids constituted 7–24% of the total fatty acids, with the greater levels present in the phospholipid fraction. Based on equivalent chain lengths (ECL) reported by other workers and results of hydrogenation of the methyl esters of the two

Table 5. Distance values calculated between fatty acid spectra of *T. philippinarum* and *T. decussatus* for triacylglycerols and phospholipids, April 1979 March 1980

	1979								1980		
	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN.	MARCH
TRIACYLGLYCEROLS											
Distance	5.8	9.6	9.9	6.1	5.9	11.1	8.1	6.0	9.9	6.4	4.8
T. decussatus - *T. philippinarum*											
PHOSPHOLIPIDS											
Distance	5.8	8.2	9.4	10.7	8.1	7.9	6.3		6.2	4.3	6.5
T. decussatus - *T. philippinarum*											

populations studied, partial and tentative identification was possible for six of these acids, comprising the majority of the 'unidentified' fractions. One of these, with an ECL of 17.62, is probably a dimethylacetal with a C18 alkyl group (Flanzy *et al.*, 1976). This molecule, presumably created during plasmalogen methylation, corresponds to the branched C18 reported by Ueda (1974) in *T. philippinarum* from Japanese waters. Another acid of ECL 20.15 is a C20:1 which is sometimes included in the C20:1 ω9 peak. This acid, when present separately, was thus included in the sum of the monoenoic acids. Four other unknowns were found to be C22 acids following hydrogenation. The ECLs of two of these corresponded to the non-methylene-interrupted diethylenes (NMID) reported in two shallow-water marine gastropods by Ackman and Hooper (1973).

The indices of reproductive activity previously published (Beninger, 1984) are presented in Fig. 4, as reference is made to these data in the following discussion.

DISCUSSION

Comparison of T. decussatus *with* T. philippinarum

The fatty acid spectra of both populations are typically marine, with a dominance of palmitic acid and of C20:5 and C22:6 polyenoic acids (Gruger *et al.*, 1964; Voogt, 1972; Yamada and Hayashi, 1975).

The distance values of Table 5 indicate that the overall differences between species in fatty acid spectra for the triacylglycerols and phospholipids are quite small (range 4–11, mean 7.5). Bottino *et al.* (1980) obtained a mean value of 5 for the fatty acid spectra of three sympatric *Penaeus* species. Conversely, two allopatric *Euphausia* species yielded a mean value of 29 (Bottino, 1975). Distance values have not previously been calculated for fatty acid spectra of bivalves, but the data of one comparative study does allow such a calculation. Watanabe and Ackman (1974) demonstrated numerous differences in the fatty acid spectra of two oyster species (*Crassostrea gigas* and *Ostrea edulis*) reared in a common habitat and fed identical algal diets. Distance values calculated from their data are approximately 15, which is twice the mean value of the two species of the present study.

In spite of the overall similarity in fatty acid spectra, several specific differences have been demonstrated when comparing the spectra of *T. decussatus* and *T. philippinarum*, although these differences are less numerous and less striking than those reported for two oyster genera by Watanabe and Ackman (1974). Taken together, these observations suggest that phylogenetically close sympatric species show great similarities in fatty acid spectra; the greater the phylogenetic distance, the more dissimilar are the fatty acid spectra. The fatty acid spectra of phylogenetically close species, even if they are of very different geographical origin (as in the present study), are quite similar when the animals are reared in a common habitat. It is not yet clear whether this similarity is due to the exploitation of a common food resource.

Relationship between fatty acid spectra and temperature

It is generally accepted that the typical marine fatty acid spectrum (with a predominance of the polyenoic C20:5 ω3 and C22:6 ω3) is an adaptation to the relatively low temperatures of the marine environment, contributing to the maintenance of cell membrane fluidity (Holland, 1978). The predominance of these two fatty acids in the phospholipids of both species is in accord with this hypothesis.

In a comparative study of numerous temperate and cold-water marine species Lewis (1962) proposed several types of adaptations to low temperature involving fatty acids. Among these were the increase in desaturation of fatty acids and the decrease in the levels of saturated acids. Farkas and Herodek (1964) demonstrated that in temperate waters these changes are seasonal, corresponding to the annual temperature cycle. The pattern is reversed during warmer seasons (Farkas, 1979), presumably an adaptation which prevents membrane hyperfluidity at warmer temperatures. It should be noted, however, that these temperature-related variations were observed in the fatty acid spectra of whole lipid extracts. No study has previously investigated such seasonal variations in the fatty acid spectra of the major lipid classes, despite the obvious relevance of such an approach.

The data of the present study clearly demonstrate the existence of such seasonal variations in the total saturated and polyenoic fatty acids of the triacylglycerols (Figs 2 and 3). In the case of the polyenoic acids these seasonal variations are mainly due to the ω3 levels (Tables 1 and 2). The total saturated and polyenoic levels of the phospholipids, however, do not present any clear seasonal variation (Figs 2 and 3). Since these seasonal variations are clearly observed only in the triacylglycerol fraction (and are thus independent of the maintenance of membrane fluidity), it is likely that their origin is dietary rather than endogenous. Several lines of evidence tend to confirm this hypothesis. Ackman *et al.* (1968) reported that the levels of polyenoic acids in unicellular Chrysophyceae were twice as great at 10°C than at 20°C. Numerous authors have shown that the triacylglycerol fatty acids of aquatic filter-feeders are quite similar to those of their diet (Jezyk and Penicnak, 1966; Lee *et al.*, 1971; Waldock and Nasciemento, 1979; Ackman, 1980; Langdon and Waldock, 1981; Gatten *et al.*, 1983), but that the fatty acid composition of the phospholipids is much less influenced by diet (Jezyk and Penicnak, 1966; Lee *et al.*, 1971; Waldock and Nasciemento, 1979; Langdon and Waldock, 1981). These observations suggest that the seasonal pattern observed in the triacylglycerol spectra of the two species studied are of dietary origin.

It might be argued that the observed variations in the levels of the triacylglycerol polyenoic fatty acids may be linked to oogenesis, as triacylglycerols constitute a major fraction of bivalve lipovitellins (see review by Holland, 1978). Indeed, increases in polyenoic fatty acids corresponding to reproductive activity have been suggested in the decapod *Penaeus japonicus* (Guary *et al.*, 1975), and a high level of polyenoic acids have been observed in cirripede eggs

(Dawson and Barnes, 1966). However, contradictory evidence has come from studies on gravid decapod females (Morris, 1973). Comparison of the triacylglycerol polyenoic levels with the indices of reproductive activity of the two populations of the present study clearly shows that polyenoic levels do not appear to be related to reproductive activity (Figs 2–4). Furthermore, the observed polyenoic variation does not correpsond to the variations in levels of triacylglycerols previously reported in these two populations (Beninger, 1984).

The absence of seasonal patterns in the phospholipid fraction suggests either different mechanisms of incorporation or differences in turnover of fatty acids in the triacylglycerols and phospholipids; and, most importantly, demonstrates the absence of any exogenous or endogenous mechanism of modifying membrane fatty acid composition in response to the seasonal temperature cycle. The results of the present study thus suggest that the changes in total lipid fatty acid composition of marine invertebrates previously attributed to endogenous responses to environmental temperature variation are more probably of dietary origin, and are confined principally to changes in the composition of the triacylglycerols.

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