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**SEASONAL VARIATIONS OF THE MAJOR LIPID CLASSES IN RELATION
TO THE REPRODUCTIVE ACTIVITY OF TWO SPECIES OF CLAMS
RAISED IN A COMMON HABITAT: *TAPES DECUSSATUS* L. (Jeffreys,
1863) and *T. PHILIPPINARUM* (Adams & Reeve, 1850)**

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Abstract: The relative and absolute levels of four major lipid classes in an indigenous and an introduced adult clam population raised in a common habitat were determined over a 74-wk period. The standard animal of each population contained a low and stable amount of the fraction containing wax esters, hydrocarbons, and sterol esters. The absolute levels of triacylglycerols and phospholipids closely followed the indices of reproductive activity. Following the summer spawning a severe negative energy balance was observed throughout the winter. During this period, both triacylglycerols and phospholipid contributed to the maintenance energy, although the phospholipids' contribution was greater in both populations.

A comparison of the seasonal variations of absolute and relative values underscores the advantages of absolute values in the calculation of lipid class levels. Although some specific differences were found in mean lipid class levels, the overall seasonal variations of the lipid classes were similar in both populations.

INTRODUCTION

Previous studies have suggested the importance of lipids in the economy of adult marine bivalves, both for the formation of gametes (Walne, 1970; Holland, 1978; Taylor & Venn, 1979), and as an energy reserve under conditions of either imposed or natural nutritional stress (Walne, 1970; Beukema & De Bruin, 1977; Beninger & Lucas, 1984). The exact nature of the lipids mobilized in these situations is uncertain, but it is known that oocytes are rich in lipids (see reviews by Gabbott, 1975; Holland, 1978) and that a large portion of adult bivalve lipids consists of phospholipids (Lubet & Le Feron De Longcamp, 1969; Gardner & Riley, 1972; Moreno *et al.*, 1976; Krishnamoorthy *et al.*, 1978).

Although numerous studies have demonstrated the seasonal variation in the biochemical composition of marine bivalves (see review by Gabbott, 1983), relatively few have documented the seasonal variation of lipid classes. None of these studies has either described the seasonal variation of tissue phospholipids or attempted to compare the seasonal variations of lipid classes in an indigenous and an introduced species raised in a common habitat. In addition, quantitative studies of such variations have been absent to date, since the data are traditionally presented in percentages, thus excluding

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evaluations of the energetic contribution of the various lipid classes, including the heretofore unspecified reserve lipids.

The present study continues the detailed investigation of the seasonal variations in the biochemical composition of an indigenous Atlantic population of the venerid clam *Tapes decussatus* L. (Jeffreys), and an introduced population of the related Indo-Pacific species *T. philippinarum* (Adams & Reeve), raised in a common habitat in the Sud-Finistère region of France. In a previous study, the seasonal variation in gross biochemical composition in relation to the reproductive cycle of the two species was reported, including that of the total lipids (Beninger & Lucas, 1984). The present work compares the seasonal evolution of four lipid classes in the two clam species during the same 16-month sampling period. A quantitative approach has been followed with the use of standard animals permitting the calculation of absolute values of the observed lipid classes. The energetic contribution of the triacylglycerols and the phospholipids could thus be evaluated during the winter period of nutritional deficiency previously reported.

MATERIAL AND METHODS

A detailed description of the sampling and extraction techniques has been given previously (Beninger & Lucas, 1984). The lipid classes were separated using a modified version of the liquid chromatography method of Nevenzel *et al.* (1965) adapted to the high phospholipid content of the lipid studied.

A 1 × 15-cm column was prepared using a slurry of Silica Gel G (Merck 7734) in hexane. The total lipids were dissolved in dichloromethane (50 µl per mg lipid) and added dropwise to 3–4 g of Silica Gel G. The dichloromethane was carefully evaporated at 60 °C using a rotary evaporator, and the impregnated silica gel was added to the hexane head of the chromatography column. The first fraction, consisting of sterol esters, waxes, and hydrocarbons, was eluted using 100 ml of 2% diethyl ether in hexane. The second fraction, composed exclusively of triacylglycerols, was eluted with 100 ml of 10% diethyl ether in hexane. The third fraction, containing sterols, mono- and diacylglycerols, and free fatty acids, was eluted with 200 ml of redistilled reagent-grade methanol. The proportion of each class was determined by weighing the residue after solvent evaporation, and multiplying this by the sum of the weights of all lipids recovered. In the case of the fourth fraction (phospholipids), the residue was first dissolved in 20 ml of chloroform and filtered, in order to eliminate traces of precipitated silica gel. Quantitative filtration was achieved with three 10-ml chloroform rinses.

This method was tested for quantitative recovery using known amounts of pure lipid standards. Thin layer chromatography of both pure standards and lipid extracts was employed to verify the separation of each fraction (Fig. 1). Using this modified method, a variety of marine lipids have since been successfully separated, including those of various body components of sea bass and salmon, as well as several teleost eggs.

The amount of each class of lipids was expressed in both relative and absolute values.

The relative value is defined as the percentage of each lipid class comprising the total lipids. The absolute value is calculated by multiplying the relative value of each lipid class by the amount, in mg, of total lipid present in a standard animal of each species (Beninger & Lucas, 1984).

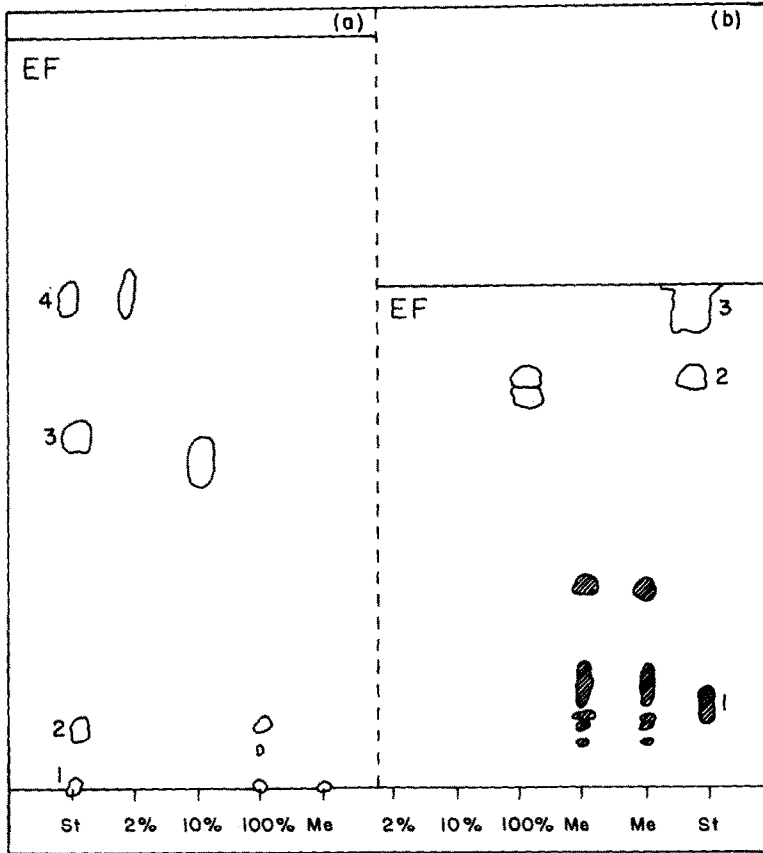


Fig. 1. Composite representation of thin-layer chromatographic verification of the purity of the four lipid classes separated by column chromatography: 2%, 10%, 100% ether fractions and methanol fraction (Me). (a), neutral lipid, TLC eluant: St, standard; 1, free fatty acid mixture + phosphatidyl choline; 2, cholesterol + diolein; 3, triolein; 4, palmityl stearate. (b), polar lipid, TLC eluant: St, standard; 1, phosphatidyl choline; 2, cholesterol; 3, triolein + palmityl stearate. Indicators: (a), iodine followed by rhodamine B-difluorescein (Jones *et al.*, 1966); (b), neutral lipid indicator series, followed by phosphorus-specific indicator (Dittmer & Lester, 1964); the hatched spots are those which reacted with the phosphorus-specific indicator. Sample, *T. decussatus* lipid extract; EF, eluant front.

Although extensively used in studies of the seasonal variation of gross biochemical composition (Giese, 1967, 1969; Ansell & Trevallion, 1967; Ansell, 1972, 1974, 1975; Ansell *et al.*, 1980; Beukema & De Bruin, 1977; Taylor & Venn, 1979; Shafee, 1981), absolute values have not as yet been applied to lipid class data. The obvious advantage

of this technique is that it allows an unbiased quantitative evaluation of the seasonal changes in lipid class composition.

The calorific conversion factors for triacylglycerols and phospholipids were established following microbomb calorimetry of each of these classes at various sampling dates for each species. Due to the low coefficients of variations (0.65 and 0.25%), the results were pooled to give a mean value for triacylglycerols of $31.06 \text{ kJ} \cdot \text{mg}^{-1}$ ($7.43 \text{ cal} \cdot \text{mg}^{-1}$), and a phospholipid value of $37.75 \text{ kJ} \cdot \text{mg}^{-1}$ ($9.03 \text{ cal} \cdot \text{mg}^{-1}$). These conversion factors are in agreement with those found in *Crassostrea virginica* by Krishnamoorthy *et al.*, (1978). It is likely that biological oxidation would actually yield a somewhat lower value for the phospholipid fraction.

A Wilcoxon rank-sum test was employed to evaluate the significance of the difference in mean levels of the four lipid fractions in each species. This test was performed assuming a theoretical pool of 100 g of total lipids.

Details of the two indices used to assess reproductive activity have been published previously (Beninger & Lucas, 1984). The first index was the temporal variation of the ash-free dry weight of the 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.

RESULTS

The indices of reproductive activity previously published (Beninger & Lucas, 1984) are presented in Fig. 2, as the subsequent discussion makes frequent reference to these data.

The results of the lipid class fractionation for the 16-month sampling period are shown in Table I. The seasonal variation of two of these lipid classes, the triacylglycerols and the phospholipids, is shown in Figs. 3 and 4, both in relative and absolute values.

The first fraction, which could include hydrocarbons, waxes, and sterol esters, is characterized by relatively low and stable absolute levels throughout the sampling period in both populations. Thin layer chromatography of this first fraction revealed only one spot with an R_f identical to that of the wax ester standard (Fig. 1). No significant difference was found between the mean levels of this first fraction in the two populations ($P > 0.05$).

The triacylglycerol fraction shows a definite seasonal variation of absolute values in both populations (Table I, Fig. 3). Furthermore, the curves of relative and absolute values are quite similar in this fraction. The maximum for *T. decussatus* occurs in mid-June 1979, with a minimum in February-March 1980, followed by a steady increase to the end of the sampling period (mid-August 1980). In *T. philippinarum*, the 1979 maximum is also observed in June, with a minimum in January-March 1980. A stepwise increase follows from March to June 1980, when a partial decrease is observed. The

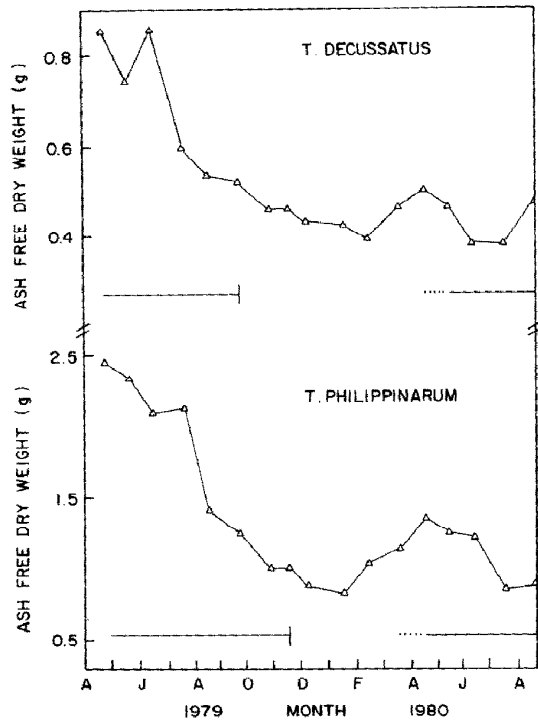


Fig. 2. Indices of reproductive activity of *T. decussatus* and *T. philippinarum*: the curve represents the ash-free dry weight (AFDW) of the standard animal, while the horizontal bar represents the period during which mature oocytes were observed in the gonad; dotted line indicates the period during which immature oocytes were observed in the gonad.

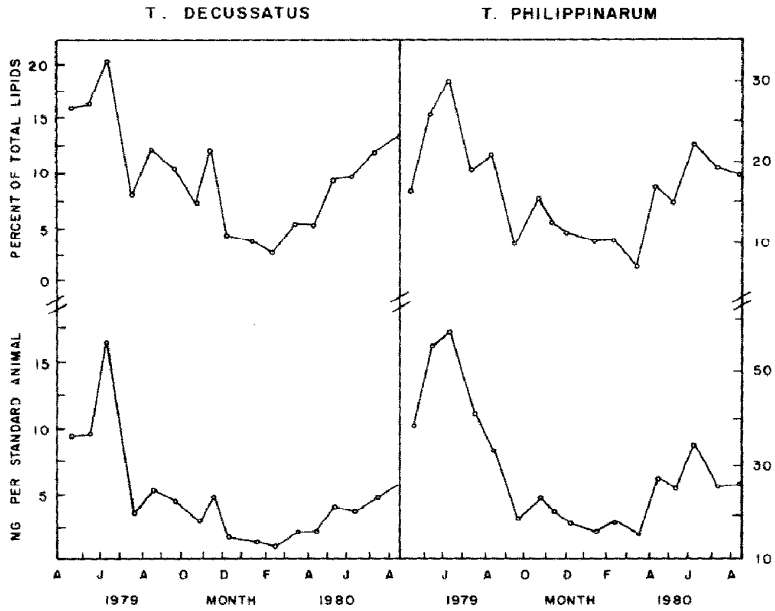


Fig. 3. Seasonal variation of triacylglycerol absolute and relative values in *T. decussatus* and *T. philippinarum*.

TABLE I

Lipid class composition of *T. decussatus* and *T. philippinarum*. April 1979–August 1980: TG, triacylglycerols; PL, phospholipids; 1, % of total lipids; 2, mg per standard animal.

	Month																
	1979						1980										
	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
<i>T. decussatus</i>																	
2% ether	1	5.36	3.69	1.48	2.59	3.37	1.79	3.23	4.52	4.30	2.73	2.10	0.51	3.68	2.64	1.79	Tr
	2	3.20	2.17	1.20	1.17	1.47	0.75	1.27	1.79	1.57	2.00	0.87	0.20	1.50	0.96	0.70	Tr
TG	1	16.16	16.40	20.58	7.92	12.36	10.63	7.48	12.20	4.46	3.96	5.64	5.37	9.46	9.85	12.02	13.53
	2	9.65	9.63	16.62	3.59	5.39	4.48	2.93	4.84	1.63	1.33	2.12	2.14	3.86	3.58	4.68	5.63
100% ether	1	16.94	18.19	21.14	21.70	17.39	17.93	26.23	19.96	16.75	18.35	18.76	22.36	17.67	20.51	22.55	17.03
	2	10.11	10.68	17.08	9.83	7.58	7.55	10.28	7.92	6.13	6.14	7.05	8.92	7.21	7.44	8.77	7.08
PL	1	64.14	61.72	59.78	67.51	66.28	69.61	63.08	63.34	74.18	71.73	73.91	71.85	69.18	66.95	63.73	69.34
	2	38.29	36.23	48.30	30.58	28.90	29.31	24.73	25.14	27.15	24.03	23.43	28.66	28.22	24.30	24.79	28.85
<i>T. philippinarum</i>																	
2% ether	1	Tr	Tr	2.00	1.79	3.62	2.92	4.15	3.93	3.42	4.28	5.31	4.33	4.60	3.81	4.83	4.83
	2	Tr	Tr	3.19	2.93	4.03	2.80	3.60	3.29	2.49	2.59	4.31	4.39	4.59	9.18	3.82	3.82
TG	1	16.53	25.92	30.31	19.07	21.08	9.80	15.65	12.72	11.37	10.33	10.45	7.02	17.28	22.61	19.66	19.66
	2	29.09	45.61	48.31	31.18	23.46	9.39	13.58	10.64	8.27	6.24	8.47	5.59	17.50	24.83	15.57	15.57
100% ether	1	14.61	10.75	15.38	16.58	13.83	20.93	19.21	23.71	18.37	19.30	18.98	22.35	18.09	17.75	15.68	15.68
	2	25.71	18.92	24.52	27.10	15.39	20.05	16.67	19.84	13.35	11.67	15.39	17.81	18.32	15.72	12.41	12.41
PL	1	68.85	63.52	51.40	62.56	61.45	66.35	60.98	59.65	66.83	66.08	65.26	68.49	60.37	62.11	59.83	59.83
	2	121.18	111.80	81.93	102.30	68.39	63.56	52.93	49.93	48.59	39.98	52.93	54.59	61.15	61.92	65.07	47.38

slight increase in absolute values between July and August suggests the possibility of a later, more complete decrease. A significant difference exists in the mean levels of triacylglycerols in the two populations (*T. decussatus*: $10.1 \text{ g} \cdot 100 \text{ g}^{-1}$ lipids; *T. philippinarum*: $16.7 \text{ g} \cdot 100 \text{ g}^{-1}$ lipids; $0.01 \leq P < 0.001$).

The absolute values of the third fraction, which could contain sterols, mono- and diacylglycerols and free fatty acids, are consistently greater than those of the triacylglycerol fraction, while this is true only during the winter months in *T. philippinarum* (Table I). Thin layer chromatography of this fraction reveals three separate, poorly-defined spots, none of which contained phosphorus (Fig. 1). In addition, the *T. decussatus* lipids contain a greater mean level of this fraction ($19.6 \text{ g} \cdot 100 \text{ g}^{-1}$ lipids) than those of *T. philippinarum* ($17.7 \text{ g} \cdot 100 \text{ g}^{-1}$ lipids; $0.01 \leq P < 0.001$).

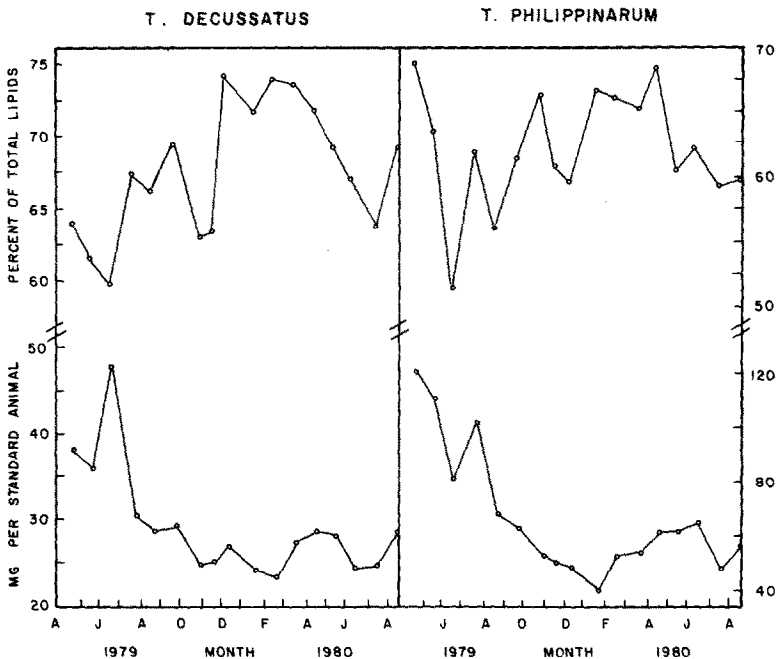


Fig. 4. Seasonal variation of phospholipid absolute and relative values in *T. decussatus* and *T. philippinarum*.

The seasonal variation and relative and absolute values of the phospholipids is shown in Fig. 4. In both populations, the relative values increase during the winter and decrease during the spring and summer, while the opposite is true for the absolute values. The *T. decussatus* lipids contain a greater mean level of phospholipids ($67.6 \text{ g} \cdot 100 \text{ g}^{-1}$ lipids) than those of *T. philippinarum* ($62.9 \text{ g} \cdot 100 \text{ g}^{-1}$ lipids; $P < 0.001$).

DISCUSSION

Thin-layer chromatography (Fig. 1) reveals that the first fraction probably contains only wax esters in any appreciable amount, and even this is quite low in the two populations studied. It is thus evident that wax esters which have been reported to constitute energy reserves in various marine invertebrates (Lee *et al.*, 1971a, b; Benson & Lee, 1972; Sargent, 1976), do not constitute energy reserves in the two species studied.

A review of the literature allows a comparison of the relative values of triacylglycerols in the two species studied with those previously reported in other bivalves (Table II).

TABLE II
Relative amounts of triacylglycerols found in various bivalve species.

Species	Amount (% total lipids)	Reference
<i>Chlamys opercularis</i>	44	Gardner & Riley, 1972
<i>Chlamys tehuelcha</i>	57-87	Pollero <i>et al.</i> , 1979
<i>Crassostrea virginica</i>	1.3-9.3	Krishnamoorthy <i>et al.</i> , 1978
<i>Crepidula fornicata</i>	26	Gardner & Riley, 1972
<i>Mesoderma mactroides</i>	≈ 25	Moreno <i>et al.</i> , 1976
<i>Mytilus edulis</i>	40-50	Lubet & Le Feron de Longcamp, 1969
<i>Mytilus edulis</i>	50	Gardner & Riley, 1972
<i>Pecten maximus</i>	13	Gardner & Riley, 1972
<i>Tapes decussatus</i>	7-31	Present study
<i>Tapes philippinarum</i>	3-21	Present study

This comparison reveals that considerable variability in triacylglycerol relative values exists within the Bivalvia. The similarity between the curves of absolute and relative triacylglycerol values suggests that the proportion of tissue triacylglycerols varies directly with the quantity of tissue. As has previously been shown with respect to total lipids in these two populations (Beninger & Lucas, 1984), the triacylglycerols show a seasonal cycle which corresponds to the synthesis and emission of gametes. The possibility of triacylglycerols constituting an energy reserve for reproductive tissues has been suggested in *Mytilus galloprovincialis* (Lubet & Le Feron De Longcamp, 1969) and in *Crassostrea virginica* (Swift *et al.*, 1980). Indeed, along with phospholipids, triacylglycerols constitute the major part of marine invertebrate (including bivalve) lipovitellins (see review by Holland, 1978).

While several authors are aware of the potential of lipids as an energy reserve in adult marine bivalves, the exact nature of these reserve lipids is uncertain. The decrease in triacylglycerol absolute values during the winter, exclusive of reproductive activity (Fig. 2), demonstrates that this lipid class may constitute an energy reserve in the two species studied, at least during the winter period of nutritional deficiency. The total lipid contribution to the maintenance energy during this period of negative energy balance

has previously been estimated at 16.3% of that contributed by all proximate reserves in *Tapes decussatus*, and 17.0% in *T. philippinarum* (Beninger & Lucas, 1984). Using the calorific conversion obtained for the triacylglycerols of the two species studied, the net contribution of triacylglycerols during this period may be estimated at 7% in *T. decussatus* and 4% in *T. philippinarum* (Table III). These results indicate that the triacylglycerols played a small but appreciable rôle as energy reserves during the winter conditions of nutrient deficiency in both populations. Such data complement the anatomical evidence of Bourcart *et al.* (1964), who observed the presence of granular adipose cells in the somatic tissue during the sexually inactive phases of adult *Mytilus galloprovincialis*, and Swift *et al.* (1980) who showed that triacylglycerol deposits in *Crassostrea virginica* were located in all tissues except the digestive gland-gonad. The latter authors also demonstrated that starved oysters contained reduced amounts of triacylglycerols.

TABLE III

Contributions of triacylglycerols and phospholipids to the maintenance energy of a standard animal of *T. decussatus* and *T. philippinarum* from the end of the 1979 spawning to the beginning of the AFDW increase in 1980: Δmg , net decrease in mg; ΔE , net energy difference; $\% \Delta E_{tot}$, ΔE as a percentage of the total net triacylglycerol and phospholipid difference.

	<i>T. decussatus</i>			<i>T. philippinarum</i>		
	Δmg	ΔE	$\% \Delta E_{tot}$	Δmg	ΔE	$\% \Delta E_{tot}$
Triacylglycerols	3.58	32.3	6.6	4.4	39.7	3.75
Phospholipids	5.88	43.7	8.92	9.95	73.9	6.97
Triacylglycerols and phospholipids	9.46	76.0	15.52	14.35	113.6	10.72

The heterogeneous nature of the third lipid fraction precludes the interpretation of its seasonal variation. Thin layer chromatography revealed that this fraction was definitely free of phospholipids and triacylglycerols, thus confirming the successful separation of these two major classes (Fig. 1).

The relative values of phospholipids found in the two populations studied may be compared with those observed in other marine bivalves (Table IV). With the exception of *Pecten maximus*, the relative values of the two *Tapes* populations are the greatest yet reported. The considerable difference between the curves of phospholipid absolute and relative values in the two populations underscores the dangers of misinterpretation when only relative values are employed. Although the proportion of phospholipids in the tissue does increase during the winter, this corresponds to a net decrease in total phospholipids present in the individuals of the two populations, since the tissue weight and organic reserves (including total lipids) decrease markedly during this period (Beninger & Lucas, 1984).

Despite the extensive literature concerning marine bivalve lipids, no previous study has been found to document the seasonal variation of the tissue phospholipids in these

organisms. The data of the present study indicate that the seasonal variation of the phospholipids in both *Tapes* populations follows quite closely that of the triacylglycerols. The maxima of spring and early summer 1979 correspond to the period of active gametogenesis, while the decrease in summer 1979 coincides with the emission of gametes (Fig. 1). It is interesting to note that Thompson (1977) observed a similar pattern for haemolymph phospholipids in *Placopecten magellanicus*.

TABLE IV
Relative amounts of phospholipids found in various bivalve species.

Species	Amount (% of total lipids)	Reference
<i>Anodonta</i> sp.	53	Gardner & Riley, 1972
<i>Chlamys opercularis</i>	44	Gardner & Riley, 1972
<i>Crassostrea virginica</i>	12.3–39.2	Krishnamoorthy <i>et al.</i> , 1978
<i>Mesoderma mactroides</i>	22–38	Moreno <i>et al.</i> , 1976
<i>Mytilus edulis</i>	44	Gardner & Riley, 1972
<i>Mytilus edulis</i>	30–40	Lubet & Le Feron de Longcamp, 1969
<i>Pecten maximus</i>	85	Gardner & Riley, 1972
<i>Tapes decussatus</i>	60–75	Present study
<i>Tapes philippinarum</i>	50–70	Present study

Phospholipids probably fulfill several functions in bivalve gametes. Besides their obvious rôle as structural elements in the cell membrane, several lines of evidence indicate that phospholipids may constitute an energy reserve in oocytes. Mature female mussels and oysters have been found to contain two to three times more phospholipids than males (Bourcart *et al.*, 1964; Krishnamoorthy *et al.*, 1978), and both phospholipids and triacylglycerols are catabolized to the same extent in *Mytilus edulis* larvae (Bayne *et al.*, 1975; Gabbott, 1975). The variation of the phospholipid absolute levels in summer 1980 follows that of the total lipids (Beninger & Lucas, 1984) and the triacylglycerols, supporting the suggestion that only partial spawning occurred during this period.

The net decrease in phospholipid absolute values during the sexually inactive winter indicates that phospholipids were catabolized during this period. The simultaneous increase in relative values suggests that the rate of catabolism was slower than that of the triacylglycerols; nevertheless, the contribution of phospholipids to the maintenance energy of both species exceeded that of triacylglycerols during this interval (Table III). Two such sources of phospholipid are possible: actual somatic reserves, such as the phospholipid droplets observed in *M. galloprovincialis* adipose cells during sexual inactivity (Bourcart *et al.*, 1964), and the catabolism of cell membranes. In the light of the decrease in protein absolute values observed during this same period (Beninger & Lucas, 1984), it is likely that most of the phospholipids mobilized originated from membrane autolysis during the winter period of nutrient deficiency.

Holland & Hannant (1976) recognized the possibility of lipids constituting an energy

reserve in adult bivalves, but indicated that the exact nature of the lipids involved was uncertain. The present study demonstrates that both triacylglycerols and phospholipids may contribute to maintenance energy in *Tapes decussatus* and *T. philippinarum*, but that the phospholipids may contribute a greater portion under severe stress conditions, after the more labile reserves have been mobilized.

The overall seasonal variation of lipid classes in the *T. decussatus* and *T. philippinarum* populations is quite similar, despite the fact that the former is an indigenous European Atlantic species, while the latter is of Indo-Pacific origin. These observations emphasize the importance of habitat in determining the physiological responses of different marine bivalve species. Certain specific differences in mean levels of three of the lipid classes have, however, been observed; *T. philippinarum* contains more triacylglycerols, while *T. decussatus* contains more of the third fraction (probably sterols) and phospholipids.

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