Lipid classes of mussel seeds Mytilus galloprovincialis of subtidal and rocky shore origin

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Abstract

The lipid class composition in juveniles of the mussel Mytilus galloprovincialis of rocky shore and subtidal origin were compared after transfer to a subtidal environment in the Ria de Arousa (northwest Spain). The experiment was conducted between November 27, 1995 and July 3, 1996. In addition to mussel origin, the influence of the different environmental parameters on the changes in lipid classes was studied. At the start of the experimental period, only the relative percentage of the triacylglycerols (energetic function) was significantly higher in the subtidal specimens. However, when the initial absolute contents were examined, the phospholipids and sterols were also significantly higher in this mussel group. Differences in the relative percentages of phospholipids and sterols were maintained until day 22 of the experiment. Our results show that during the first 36 days of the experimental period the mussel origin participated significantly in the model explaining the variance of triacylglycerols, phospholipids and sterols. These results suggest the initial differences in content and relative percentages of the lipid classes studied are possibly linked to the contrasting environmental conditions in which the two mussel groups had previously developed (subtidal and rocky shore habitats). In contrast, 50 days into the experiment the origin term did not participate in the model of variance of these lipid classes. These results in turn suggest that during the course of the investigation the mussel seeds of rocky shore origin were able to exploit the available food resources in the subtidal habitat. Based on these results, the influence of mussel origin and environmental parameters on the changes in lipid classes of both mussel groups is discussed.

Keywords: Environmental conditions; Lipid classes; Mussel; Mytilus galloprovincialis; Subtidal and rocky shore origins

1. Introduction

Rodhouse et al. (1984a,b) have observed that quantitative and qualitative differences of the available food in rocky shore and subtidal zones differentially affect the growth and reproduction rates of mussels. Furthermore, one of the conditions which has the highest influence on the mussel energy reserves are periods of exposure to air, due to the fact at such times the organisms cannot feed. Consequently, these periods would have a similar effect to starvation (Hummel et al., 1989). Accordingly, a decrease in triacylglycerol values has been observed in the larval stages of some marine invertebrates and bivalve juveniles subjected to conditions of nutritional stress (Fraser, 1989; Caers et al., 2000).

Changes occurring in mussel lipid reserves have been observed to be mainly influenced by reproduction (Pieters et al., 1979; Pollero et al., 1979; Lubet et al., 1986; Chu et al., 1990) and/or by nutrition (De Moreno et al., 1976, 1980; Fernández-Reiriz et al., 1998; Okumus and Stirling, 1998). Seasonal variation in lipid content is mainly caused by fluctuations in triacylglycerols and not phospholipids (Trider and Castell, 1980; Pazos et al., 1996, 1997). This is due to the fact that phospholipids, mainly serving structural-type functions, are maintained virtually constant over the year (Gadner and Riley, 1972; Swift, 1977; Pazos et al., 1997). On the other hand, triacylglycerols are accumulated as reserve energy. For this reason, low levels of triacylglycerols have been considered an indicator of low nutritional state (Swift et al., 1980). In contrast, polar lipids are less influenced and maintained practically constant over the year (Gardner and Riley, 1972; Swift, 1977; Beninger and Stephan, 1985; Pazos et al., 1997; Soudant et al., 1999). This maybe due to the fact that they serve in various structural functions (Nes, 1974; Pollero et al., 1979; Trider and Castell, 1980; Beninger, 1984; Pazos et al., 1996).

The initial aquacultural demand for seeds of the mussel Mytilus galloprovincialis in the Galician rias was met by extracting mussels located in the intertidal rocky shore zone. With the growth of industrial aquaculture, this seed source became insufficient to supply the 7500 tons required at present (Pérez-Camacho et al., 1995) Accordingly, Pérez-Camacho et al. (1995) observed a higher growth rate in seed of collector origin,

whereas Fuentes et al. (1998) noted a greater survival rate in seed of rocky shore origin. In light of this finding, a series of studies were brought forward with the aim of determining the nature of the factors which caused these differences.

Pérez-Camacho et al. (1995) attributed their findings to the higher condition index and the previous adaptation of the collector mussels to the culture conditions of permanent immersion. Correspondingly, physiological differences between mussels distributed in habitats with contrasting environmental conditions have been reported (Okumus and Stirling, 1994; Labarta et al., 1997).

In the present study, the changes in the different lipid classes of M. galloprovincialis seed from subtidal and rocky shore origin were compared with the aim of ascertaining the influence of habitat conditions on the biochemical changes within these organisms.

2. Materials and methods

2.1. Experimental design

The experiment was carried out from November 27, 1995 to July 3, 1996. So, the experimental period commenced in winter in order to minimize any possible advantages that subtidal seed may have over rocky shore seed, as a result of being previously adapted to cultivation in the subtidal conditions (Babarro et al., 2000a,b,c). Individuals used in this study were taken from two habitats in the Arosa Ria of different ecological characteristics, from rocky shore zone and from collector ropes (subtidal environment) suspended from a mussel raft. Both groups of seed were from the previous spring–summer spawning and therefore belonged to the same year class. Additionally, the sampling locations for both populations were 2 km away from each other. The initial mean size of subtidal and rocky shore groups was 22.55 (sd = 1.55) and 19.02 mm (sd = 1.93), respectively, whereas the total dry weight was 0.36 (sd = 0.06) and 0.27 g (sd = 0.06), respectively. So, at the outset, no significant differences in size and initial mass of both mussels groups were noted (ANOVA, $P > 0.05$).

The study site, the conditions in which individuals from both mussel groups were maintained and the sampling periods, with regards to both individuals and environmental parameters, have been described previously by Babarro et al. (2000a).

2.2. Sample treatment

For each survey three sub-sets of mussels (n = 3), each comprising 30 individuals, were taken at random from both mussel groups, thus making a total of 90 individuals per mussel group. The soft tissues of the individuals of each sub-sample were separated, freeze-dried at -70ºC under a vacuum of 0.018 mbar and stored at -70ºC. Prior to the development of the biochemical analyses, the tissues were pulverized using a model 6 ‘‘Fritsch’’ pulverisette, and homogenized with water in an ultrasonic vibrator Sonifier 250.

2.3. Analysis of lipids

The lipids were extracted following the method of Bligh and Dyer (1959), modified by Fernández-Reiriz et al. (1989). The data for the different classes of lipids are expressed in absolute values (mg individual^{-1}) and in percentages relative to the organic material (% total lipid).

The different lipid classes were determined by thin layer chromatography (TLC) employing silica gel plates (Merck 16486) of dimensions 20 x 20 mm and thickness 0.25 mm. Exposure of the chromatographic stain followed the method described by Freeman and West (1966). The samples were placed on the plates by means of an automatic applier for TLC (Camag 27220) and developed in a solution of 10% $CuSO_4$ and 0.85% H_3PO_4 preheated to 180ºC (Bitman and Wood, 1982). Cholesterol palmitate, cholesterol, palmitic acid and tripalmitin (Sigma) were employed as quality standards for the quantitative analysis of the sterol esters + wax, sterols, free fatty acids (FFA) and triacylglycerols. With regards to phospholipids, a standard from the mussel M. galloprovincialis was used. The developed plates were read with a Shimadzu CS9000 densitometric-scanner fitted with a monochromatic bulb of 370 nm by 0.4 x 0.4 mm. The scanner read the stain in zigzag (complete migration), from a base line automatically graduated to zero (0).

2.4. Statistical analysis

Differences of the lipid classes between subtidal and rocky shore mussel seed were analized by ANOVAs. Relative percentage of the lipid classes were previously arcsine transformed (Zar, 1984) and Bartlett test of homogeneity of variance was applied to the data.

To study the influence of environmental parameters on the variability in the subtidal and rocky shore mussel seed lipid classes, a ‘‘multivariate stepwise regression’’ was performed. In this analysis, the ‘‘origin’’ factor is attributed to a qualitative factor (dummy) in such a way that the subtidal mussels are assigned a value of 0 (zero) and those of rocky shore a value of 1 (one). In all cases, the values expressed as relative percentage of the different lipid classes were previously transformed to the arcsine to obtain maximum r^2 values (Zar, 1984).

3. Results

3.1. Environmental parameters

With the advance of winter a sustained decrease in water temperature was observed until reaching a minima (12.5 BC) (Fig. 1A). Thereafter, a sustained increase of temperature during spring was noted, until peaking in June (16.3 BC). Chl-a presented relatively low values during the winter (Fig. 1B), with minimal concentrations (0.61 μg l^{-1}): however, chl-a increased shortly thereafter, reaching maximum values during spring (3.71 μg l^{-1}). With regards to seston between late November and early February (Fig. 1C) a series of fluctuations occurred, notably the TPM, POM and PIM maxima in early January (2.56, 1.00 and 1.29 mg l^{-1}, respectively). Following these augmented concentrations, two new increments in TPM and POM were observed in February (1.34 and 0.57 mg l^{-1}, respectively) and in April (1.381 and 0.64 mg l^{-1}, respectively). Similar events to those described for seston also occur in the particulate volume (Fig. 1D) with an emphatic peak in April (1.66 mm^3). Accordingly, three peaks can be observed in the particulate volume, the first corresponding with the seston maxima at the start of January, and the following two peaks corresponding to those observed in chl-a during

spring. With regards to food quality, two clear periods can be discerned in Q_2 (Fig. 1E). The first interval between late 100 November and mid February (winter) was characterised with values generally above 0.6, and the second in spring, had values generally below 0.6. These two clearly defined intervals are reflected in the evolution of the ratio chl-a/POM (Fig. 1F).

3.2. Variations of lipid classes

At the start of the experimental period, significantly higher relative percentages (ANOVA, $P < 0.001$) were only observed in the triacylglycerol lipid class of the subtidal mussels (Table 1). The phospholipids, sterols and FFA were significantly higher in the mussels of rocky shore origin (ANOVA, $P < 0.001$, 0.05 and 0.01, respectively). Absolute content of the phospholipids and triacylglycerols were significantly higher (ANOVA, $P < 0.05$ and 0.001, respectively) in the mussels of submareal origin (Table 2).

With regards to the time steps of the observed differences in the relative percentages of phospholipids and sterols of both mussel groups from the start of the experimental period, statistical differences were maintained until day 22, whereas the triacylglycerols were steady until day 15. In constrast, these differences were also maintained in FFA for the first 15 days, but alternating between both groups of mussels, with the result that no defined trend was observed. With regards to differences in lipid content, in the triacylglycerols were maintained to day 22, whereas statistical differences in phospholipid content were only observed at the start of the experiment. On day 36, significantly higher contents of phospholipids, sterols and FFA (ANOVA, $P < 0.05$) again arose in the subtidal mussels, and subsequently (day 50) differences disappeared and were only observed in isolated samplings, with the result that no defined standard could be achieved.

The sterol esters + wax were present at trace levels during the experimental period in both mussels groups (Tables 1 and 2).

3.3. Influence of environmental parameters on the variations in lipid classes

The multiple regression analysis applied to the period covering the first 36 days of the study (Table 3), established that the mussel origin (rocky shore or subtidal) significantly explained almost half of the total variance observed in the phospholipids (39.2%). Furthermore, the coefficient was positive, which demonstrated that the rocky shore mussels showed higher values than the subtidal mussels. Temperature and TPM further increased the percentage explanation of the variance in phospholipids up to 77.3% and 84.0%, respectively. With regards to sterols, the model estimated from the multiple analysis showed that temperature alone explains 61.7% of the variance, whereas the chl-a/POM and the mussel origin raised the explanation to 75.4% and 86.1%, respectively. Water temperature contributed more than half (53.1%) of the explanation for variance in triacylglycerols, which further increases to 72.6% and 89.1% by the participation of the mussel origin and TPM terms, respectively.

Changes were noted in the model from day 50 due to the fact that the mussel origin did not participate in explaining the variance of any of the lipid classes studied (Table 4). Furthermore, chl-a/POM now explained more than half (55.1%) of the phospholipid variance over this period, which was raised to 67.7% by temperature. With regards to sterols, the model obtained from the regression analysis showed that chl-a/POM explained the major part (74.3%) of the variance within this lipid group and the combination of temperature and TPM slightly increases the explanation up to 82.0% and 83.8%, respectively. In this case, temperature, TPM and chl-a/POM presented a negative coefficient, which indicates their inverse relationship with this lipid class. The ratio chl-a/POM for triacylglycerols was the only environmental parameter contributing to the explanation of the variance in this lipid component. The role of chl-a/POM was sufficient to explain alone more than half of the variance (56.5%). Moreover, the coefficient was positive and demonstrated a direct relationship between phytoplanktonic food availability and increases in this lipid class during the spring–summer seasonal transformation.

4. Discussion

The greatest initial difference in the absolute content of the different lipid classes was observed in the triacylglycerols, whereby the subtidal mussels had a value 9.25 times greater than the rocky shore mussels. In contrast, phospholipids in the subtidal mussels

were only 1.33 times greater than in rocky shore mussels. Further, the differences in triacylglycerols were maintained for a longer period (22 days) than for the phospholipids and the FFA. These trends can be explained bearing in mind that in other studies which have focussed on the different life stages of various marine bivalves, the triacylglycerols and phospholipids were the principal lipid classes (Delaunay et al., 1992; Abad et al., 1995; Pazos et al., 1996, 1997; Fernández-Reiriz et al., 1998). However, whereas phospholipids present a structural-type function (Giese, 1966; Beninger and Lucas, 1984), triacylglycerols present an energy reserve function (Holland, 1978; Fraser, 1989). Consequently, other workers have observed that seasonal changes in the relative percentage of total lipids of some bivalve species are principally caused by fluctuations in the triacylglycerols whereas the phospholipids are maintained relatively constant (Trider and Castell, 1980; Pazos et al., 1996, 1997).

In contrast to the triacylglycerols, the absence of significant differences in the initial absolute content of sterols could be related to the independence displayed by this lipid group towards the ambient nutritional conditions (Sasaki et al., 1986), or to the fact that their respective biosynthesis or digestion and incorporation proceeds at a relatively low rate (Teshima et al., 1987). Sterols form part of cell membranes and contribute a constant proportion in cell structure (Trider and Castell, 1980) and thus fluctuations will be minimal.

With regards to temporal variability, we can note that with respect to phospholipids, sterols and triacylglycerols, the same differences are prolonged up to 15 days from experimental commencement. On the other hand, the changes in triacylglycerols were more drastic, especially in the subtidal mussels where the loss–gain balance of absolute content (first 36 days) was -2.18 mg, whereas in the rocky shore mussels it was -0.50 mg. These differences demonstrate a relative higher metabolic expenditure of the subtidal mussels and agree with those obtained by Babarro et al. (2000c) who found that M. galloprovincialis mussel seed of subtidal origin showed higher oxygen consumption rates than the rocky shore mussels during the first 2 weeks of the experiment. Accordingly, these authors suggested that these differences could be attributed to a lower metabolic rate for the mussels distributed in the rocky shore zone, which would lead to a reduction in energy expenditure. Accordingly, it has been shown that the individuals subjected to frequent periods of exposure to air have a lower metabolic rate

(Storey and Storey, 1990), and can be considered as a compensating trait for the lower feeding or energy acquisition time (Shick et al., 1988).

Apart from the differential effect that the metabolic rate could cause in both groups of specimens, lipid levels in marine bivalves have also been observed to be controlled by other factors, among others: reproduction (Chapat et al., 1967; Pieters et al., 1979; Kluytmans et al., 1985; Napolitano and Ackman, 1992; Pazos et al., 1997), species sex (Lubet et al., 1986), temperature (Sastry, 1968), diet (Beninger and Stephan, 1985; Napolitano et al., 1992; Fernández-Reiriz et al., 1996, 1998) and periods of starvation (Fraser, 1989; Caers et al., 2000).

Our results indicate that the initial lipid class values of the rocky shore specimens were influenced by periods of air exposure. As described above, exposure has been compared with the aforementioned periods of starvation (Hummel et al., 1989). Consequently, these mussels presumably relied on their energetic reserves to compensate for lower food availability. In this scenario, the lipid classes of mainly energetic composition, for example triacylglycerols, will supply the energy. In the larval (Holland, 1978; Gallager and Mann, 1986; Gallager et al., 1986) and juvenile stages (Caers et al., 2000) a decrease in triacylglycerols has been clearly observed in some bivalves subjected to starvation phases.

With the aim of ascertaining whether the origin factor of both mussel groups exercised any influence on the observed values of the different lipid classes studied here, the experimental period was divided into two sub-periods. The first period embraced day 1–36, and the second from day 50 until experiment termination. Prior to selecting this temporal criterion, the ANOVA results were examined. Consideration was given to the absolute values and the relative percentages of the different lipid classes and, more importantly, to the results obtained by Babarro (1998) and Babarro et al. (2000b,c) in which the differences in the physiological rates of both seed groups were observed to recede, with absorption efficiency differences being the last to disappear after 64 days had elapsed.

Accordingly, employing the data obtained from the multiple regression results concerning the influence of the different environmental parameters and seed origin on

the variations in the different lipid classes, we can highlight the following resulting facets. In the first 36 days of the experimental period, the mussel origin explained near to or greater than 50% of the total explained by each of the phospholipid, triacylglycerol and sterol models. These results agree with the already established concept which proposes that the lipids clearly reflect the biochemical composition of the environmental conditions where bivalve development occurs (De Moreno et al., 1980; Napolitano and Ackman, 1992; Napolitano et al., 1992; Fernández-Reiriz et al., 1996). On the other hand, temperature contributes to the explanation of variance in the phospholipids, sterols and triacylglycerols, but in contrast to the latter, the relationship is inverse for the first two lipid classes as indicated by the negative coefficient. In general terms, this trend stems from the parallel increase of phospholipids and sterols with temperature decreases during the autumn–winter transition. Conversely, the negative values observed in the coefficient of the mussel origin and triacylglycerols is due to lower relative percentages observed in the rocky shore mussels. chl-a/POM only participates in the explanation of observed variance for the sterols, but the inverse relationship illustrates the participation of non-phytoplanktonic POM as an alternative source of food during the transitional period between autumn and winter. The higher variability of POM is also confirmed in the seston changes (see Fig. 1C).

As previously observed, the environmental parameters which exercise an important influence on the evolution of the phospholipids, sterols and triacylglycerols during the first 36 days of the experimental period were temperature and seston, specifically TPM and POM. Accordingly, this period was characterised by a sustained lower temperature, brisk fluctuations of TPM and POM and low concentrations of chl-a. These trends are due to the advance of winter conditions, characterised by a decrease in solar irradiation, which lead to decreases in temperatures and phytoplankton abundance. Furthermore, the high rates of resuspension observed during this period are attributed to strong storm events (Babarro et al., 2000a).

When the period from day 50 to the end of the experiment was analysed, the multiple regression analysis showed that mussel origin was not a significant statistical term of lipid class variance. This suggests that during the first 36 days the rocky shore mussel seeds had an advantage by having a lower metabolic rate when food availability offered by the subtidal habitat was low but constant, as indicated by Babarro et al. (2000c).

Furthermore, in view of the fact that possible adaptations in the rocky shore mussels are reflected in the energy reserves of both mussels groups, it would seem that these changes are closely coupled with the parameters related to energy acquisition from the environment. Accordingly, it has been shown that by placing specimens of the mussel M. galloprovincialis of rocky shore and subtidal origin in the same environment (subtidal) the differences observed in the rates of clearing and ingestion (Babarro et al., 2000b), absorption (Labarta et al., 1997) and of food absorption efficiency (Babarro, 1998) disappear at 15, 36 and 64 days (respectively) after experimental initiation. Consequently, as shown in our results, a 50-day period (time after which no further significant differences in the absolute values of the lipid classes of both mussel groups were observed) would be sufficient for the rocky shore mussels to display strategies of energy compensation, under the experimental conditions.

In view of the fact that none of the environmental parameters participate significantly in explaining the variance, it is evident that the modifications in FFA obey different factors to those studied. Accordingly, since the presence of FFA has previously been associated with the hydrolysis or breakdown of the acylglycerols (Caers et al., 2000) it is likely that changes in FFA obey an endogenous factor, such as the catabolic and anabolic processes required for biosynthesis and energy production. Nevertheless, with regards to bivalve molluscs Napolitano et al. (1988) considered that FFA are a class of characteristic lipids and not the results of breakdown or hydrolysis of the triacylglycerols.

In contrast to the absence of explanation of mussel origin by the model after day 50, the ratio chl-a/POM began to play a greater role in the explanation of the different lipid classes, with the exception of the FFA. chl-a/POM alone explains more than half of the variance obtained in the phospholipid, sterols and triacylglycerol lipid classes, with a negative relationship towards the phospholipids and sterols and positive with the triacylglycerols. This implies that the sustained increase in triacylglycerols, with the onset of spring is due to the parallel availability of phytoplanktonic food. This increase could result from direct incorporation of the lipids contained in the phytoplankton, as reported in a number of studies under natural conditions (De Moreno et al., 1980; Besnard et al., 1989) and in the laboratory (Delaunay et al., 1992; Fernández-Reiriz et al., 1998, 1999; Labarta et al., 1999) or, on the other hand, by transformation of the

glycogen previously acquired from the phytoplankton and accumulated in the organs as lipid reserves (Vassallo, 1973; Waldock and Holland, 1979; Barber and Blake, 1991).

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Fig. 1. Fluctuations of the environmental parameters temperature (A), chlorophyll a (B), seston (C), particulate volume (D), food quality (Q_1 POM/TPM and Q_2 POM/mm^3) (E) and chl-a/POM (F), over the experimental period.

Table 1

Means and standard deviations of the relative percentage of the following lipid classes of mussels of subtidal and rocky shore origin: phospholipids, sterols, sterols esteres + waxes, triacylglycerols and free fatty acid, n = 3

Samples	Dates (days)	Origin	Phospholipids (% lipids)	Sterols (% lipids)	Sterols esters + waxes (% lipids)	Triacylglycerols (% lipids)	Free fatty acids (% lipids)
1st	27/11/95 (0)	Subtidal	53.35 ± 0.56* * *	5.63 ± 0.25 *	traces	34.96 ± 1.34* * *	6.06 ± 0.53 * *
		Rocky shore	72.83 ± 0.22	8.43 ± 0.35	traces	6.54 ± 0.51	12.20 ± 0.38
2nd	05/12/95 (8)	Subtidal	59.25 ± 0.94 * *	7.16 ± 0.53 *	traces	19.78 ± 0.31 * *	13.82 ± 0.91* * *
		Rocky shore	71.71 ± 0.86	10.56 ± 0.42	traces	14.01 ± 0.63	3.73 ± 0.19
3rd	13/12/95 (15)	Subtidal	61.52 ± 2.23 *	9.69 ± 0.71 *	traces	26.49 ± 2.84 *	2.29 ± 0.10 * *
		Rocky shore	72.62 ± 0.15	10.78 ± 0.20	traces	11.31 ± 0.28	5.29 ± 0.24
4th	20/12/95	Subtidal	71.95 ± 0.53 *	13.17 ± 0.24	traces	9.53. ± 1.13	5.36 ± 1.34

	(22)			*			
		Rocky shore	77.09 ± 1.10	14.44 ± 0.62	traces	traces	8.46 ± 0.48
5th	03/01/96 (36)	Subtidal	76.03 ± 1.28	14.02 ± 0.62	traces	traces	9.95 ± 0.66
		Rocky shore	77.67 ± 0.61	14.88 ± 0.14	traces	traces	7.45 ± 0.46
6th	17/01/96 (50)	Subtidal	73.48 ± 0.51	13.62 ± 0.24	traces	traces	12.89 ± 0.75
		Rocky shore	71.67 ± 0.20	12.38 ± 1.27	traces	traces	15.95 ± 1.25
7th	31/01/96 (64)	Subtidal	71.85 ± 2.45	11.24 ± 0.41	traces	traces	16.91 ± 2.03
		Rocky shore	77.44 ± 0.72	10.51 ± 0.41	traces	traces	12.05 ± 0.73
8th	15/02/96 (80)	Subtidal	57.94 ± 1.14	5.12 ± 0.55	traces	25.66 ± 0.07	11.27 ± 0.66
		Rocky shore	55.94 ± 1.44	5.33 ± 0.78	traces	25.29 ± 2.70	13.44 ± 0.47
9th	28/02/96	Subtidal	68.07 ± 0.43	8.89 ± 0.04	traces	17.48 ± 0.74	5.56 ± 0.26

	(95)						
		Rocky shore	65.12 ± 2.19	8.75 ± 0.31	traces	18.15 ± 2.99	7.98 ± 1.11
10th	13/03/96 (110)	Subtidal	53.73 ± 0.83	7.29 ± 0.06 *	traces	29.73 ± 0.98	9.25 ± 0.10
		Rocky shore	51.09 ± 1.59	6.13 ± 0.24	traces	34.18 ± 1.05	8.59 ± 0.30
11th	27/03/96 (125)	Subtidal	46.73 ± 0.81	4.79 ± 0.15	traces	41.96 ± 0.87	6.52 ± 0.09
		Rocky shore	49.39 ± 2.13	4.97 ± 0.28	traces	37.77 ± 3.38	7.86 ± 0.98
12th	10/04/94 (140)	Subtidal	48.49 ± 2.28	3.97 ± 0.10	traces	39.55 ± 1.74	7.98 ± 0.45
		Rocky shore	43.34 ± 0.68	3.85 ± 0.34	traces	44.97 ± 0.45	7.84 ± 0.11
13th	24/04/96 (155)	Subtidal	43.26 ± 0.19	3.48 ± 0.18	traces	45.94 ± 0.14	4.32 ± 0.23
		Rocky shore	40.46 ± 1.26	3.38 ± 0.40	traces	51.69 ± 2.04	4.47 ± 0.38
14th	05/06/96	Subtidal	42.27 ± 1.59	4.13 ± 0.32 *	traces	50.46 ± 1.50	3.14 ± 0.41

	(197)						
		Rocky shore	41.53 ± 1.72	2.35 ± 0.39	traces	52.76 ± 2.82	3.36 ± 0.71
15th	03/07/96 (228)	Subtidal	45.01 ± 2.31	4.58 ± 0.21	traces	46.83 ± 3.06	3.57 ±0.54
		Rocky shore	50.16 ± 0.17	4.91 ± 0.32	traces	42.50 ± 0.39	2.43 ± 0.24

* P < 0.05.

** P < 0.01.

*** P < 0.001.

Table 2

Means and standard deviations of the content (mg mussel^{-1}) of the following lipid classes of mussels of subtidal and rocky shore origin: phospholipids, sterols, sterols esteres + waxes, triacylglycerols and free fatty acid

Samples	Dates (days)	Origin	Phospholipids (mg mussel^{-1})	Sterols (mg mussel^{-1})	Sterols esters + waxes (mg mussel^{-1})	Triacylglycerols (mg mussel^{-1})	Free fatty acids (mg mussel^{-1})
1st	27/11/95 (0)	Subtidal	2.26 ± 0.09 *	0.24 ± 0.02	traces	1.48 ± 0.01* * *	0.26 ± 0.03 *
		Rocky shore	1.69 ± 0.08	0.21 ± 0.04	traces	0.16 ± 0.01	0.30 ± 0.05

2nd	05/12/95 (8)	Subtidal	2.44 ± 0.71	0.29 ± 0.06	traces	0.76 ± 0.12*	0.57 ± 0.15*
		Rocky shore	1.66 ± 0.42	0.24 ± 0.05	traces	0.32 ± 0.06	0.09 ± 0.03
3rd	13/12/95 (15)	Subtidal	3.61 ± 0.53	0.57 ± 0.06	traces	1.46 ± 0.29 *	0.14 ± 0.03
		Rocky shore	3.18 ± 0.34	0.47 ± 0.04	traces	0.50 ± 0.06	0.23 ± 0.01
4th	20/12/95 (22)	Subtidal	2.99 ± 0.27	0.55 ± 0.06	traces	0.40 ± 0.08*	0.22 ± 0.04
		Rocky shore	2.53 ± 0.63	0.47 ± 0.09	traces	traces	0.28 ± 0.05
5th	03/01/96 (36)	Subtidal	3.99 ± 0.29 *	0.73 ± 0.01**	traces	traces	0.52 ± 0.01**
		Rocky shore	1.79 ± 0.26	0.34 ± 0.06	traces	traces	0.17 ± 0.04
6th	17/01/96 (50)	Subtidal	3.54 ± 1.85	0.65 ± 0.34	traces	traces	0.55 ± 0.26
		Rocky shore	2.94 ± 0.47	0.50 ± 0.03	traces	traces	0.66 ± 0.16

7th	31/01/96 (64)	Subtidal	3.51 ± 0.61	0.55 ± 0.06*	traces	traces	0.82 ± 0.02**
		Rocky shore	2.37 ± 0.25	0.28 ± 0.02	traces	traces	0.33 ± 0.06
8th	15/02/96 (80)	Subtidal	4.05 ± 0.14	0.36 ± 0.03	traces	1.79 F 0.03	0.79 ± 0.04
		Rocky shore	4.20 ± 1.31	0.41 ± 0.17	traces	1.86 F 0.34	1.01 ± 0.33
9th	28/02/96 (95)	Subtidal	6.50 ± 0.54	0.85 ± 0.07	traces	1.67 F 0.06	0.53 ± 0.07
		Rocky shore	6.20 ± 0.64	0.83 ± 0.03	traces	1.72 F 0.16	0.76 ± 0.16
10th	13/03/96 (110)	Subtidal	11.51 ± 3.94	1.56 ± 0.52	traces	6.32 F 1.88	1.98 ± 0.67
		Rocky shore	9.24 ± 3.78	1.09 ± 0.38	traces	6.11 F 2.15	1.54 ± 0.53
11th	27/03/96 (125)	Subtidal	13.92 ± 2.53	1.43 ± 0.24	traces	12.55 F 2.75	1.95 ± 0.41
		Rocky shore	14.53 ± 3.57	1.47 ± 0.37	traces	10.96 F 1.26	2.33 ± 0.76

12th	10/04/94 (140)	Subtidal	28.47 ± 2.01	2.34 ± 0.33	traces	23.34 ± 1.76	4.71 ± 0.81
		Rocky shore	23.62 ± 0.82	2.10 ± 0.15	traces	24.50 ± 0.23	4.27 ± 0.14
13th	24/04/96 (155)	Subtidal	27.73 ± 4.17	2.22 ± 0.23	traces	29.47 ± 4.64	4.72 ± 0.88
		Rocky shore	24.99 ± 3.41	2.10 ± 0.47	traces	31.81 ± 2.11	4.77 ± 0.53
14th	05/06/96 (197)	Subtidal	43.46 ± 5.47	4.23 ± 0.37	traces	52.17 ± 10.04	3.27 ± 0.95
		Rocky shore	52.36 ± 18.50	3.02 ± 1.41	traces	65.53 ± 17.23	4.35 ± 2.22
15th	03/07/96 (226)	Subtidal	46.62 ± 4.01	4.74 ± 0.44	traces	48.89 ± 9.85	3.60 ± 0.05
		Rocky shore	43.41 ± 5.12	4.26 ± 0.80	traces	36.76 ± 4.13	2.12 ± 0.47

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 3

Stepwise multiple regression of the different classes of lipids (phospholipids, sterols and triacylglycerols), of the mussel M. galloprovincialis of sub-tidal and rocky shore origins with environmental parameters, during the period between 1st and 5th samples (autumn–winter)

Parameters	Coeficients	SE	F-ratio	r^2	P
Phospholipidos					
Constant	96.998				
Origin	6.096	0.626	39.261	0.392	< 0.001
T	-3.047	-0.495	20.053	0.773	< 0.001
TPM	1.843	0.287	6.769	0.840	< 0.01
$r^2 = 0.840$; n = 20, $F_{3,16} = 132.856$; $P < 0.001$					
Sterols					
Constant	54.247				
T	-2.244	-0.631	39.098	0.617	< 0.001
chl-a/POM	-2.029	-0.401	15.758	0.754	< 0.001
Origin	1.839	0.327	12.324	0.861	< 0.01
$r^2 = 0.861$; n = 20, $F_{3,16} = 33.022$; $P < 0.001$					
Triacylglicerols					
Constant	-96.431				
T	8.489	0.537	34.746	0.531	< 0.001
Origin	-11.027	-0.441	28.674	0.726	< 0.001
TPM	-7.391	-0.449	24.298	0.891	< 0.001
$r^2 = 0.891$; n = 20, $F_{3,16} = 927.464$; $P < 0.001$					

T = temperature, POM = Particulate organic material, TPM = Total particulate material.

Table 4

Stepwise multiple regression of the different classes of lipids (phospholipids, sterols and triacylglycerols), of mussel M. galloprovincialis of subtidal and rocky shore origins with environmental parameters, during the period between 6th and 15th samples (winter– spring–summer)

Parameters	Coeficient	SE	F-ratio	r^2	P

Phospholipids					
Constant	118.831				
chl-a/POM	-3.836	-0.519	22.207	0.551	< 0.001
T	-4.303	-0.419	14.473	0.677	< 0.001
$r^2 = 0.677$; n = 40, $F_{2,37} = 38.862$; $P < 0.001$					
Sterols					
Constant	49.321				
chl-a/POM	-2.304	-0.643	61.451	0.743	< 0.001
T	-1.931	-0.388	21.055	0.820	< 0.001
TPM	-1.752	-0.147	4.164	0.838	< 0.05
$r^2 = 0.838$; n = 40, $F_{3,36} = 137.038$; $P < 0.001$					
Triacyglycerols					
Constant	-4.794				
chl-a/POM	12.533	0.752	49.364	0.565	< 0.001
$r^2 = 0.565$; n = 40, $F_{1,38} = 54.039$; $P < 0.001$					

T = temperature, POM = Particulate organic material, TPM = Total particulate material.