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Gonad development and fatty acid composition of *Patella depressa* Pennant (Gastropoda: Prosobranchia) populations with different patterns of spatial distribution, in exposed and sheltered sites

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Abstract

The present study examines the effect of shore exposure on the feeding performance (assessed by fatty acid analyses of the whole body) and gonad condition (stage of development and gonad somatic index, GSI) of *Patella depressa* populations. Male and female limpets were collected at exposed and sheltered sites, during winter and summer. The population at the exposed site was at a more advanced stage of gonad development, with a higher dispersion of gonad stages, both in winter and summer. Additionally, limpets from the exposed site, particularly the males, presented a higher GSI than the corresponding stage in the sheltered site. The quantitatively most important fatty acids were the saturated fatty acids (SFA) 16:0, 14:0, and 18:0, the monounsaturated fatty acids (MUFA) 18:1($n-7$), 18:1($n-9$), 16:1($n-7$) and 20:1($n-9$) and the polyunsaturated fatty acids (PUFA) 20:5($n-3$) and 20:4($n-6$). Females had a significantly higher fatty acid methyl esters (FAME) content (in summer and winter) and higher amounts of SFA and MUFA (in

Abbreviations: ARA, arachidonic acid, 20:4($n-6$); DHA, docosahexaenoic acid, 22:6($n-3$); EPA, eicosapentaenoic acid, 20:5($n-3$); FAME, fatty acid methyl esters; HUFA, highly unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

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summer), which points to a higher degree of storage of neutral lipids in this sex. Male and female limpets at the exposed site had a significantly higher FAME, SFA, MUFA, PUFA and highly unsaturated fatty acids (HUFA) content than the corresponding sex in the sheltered site in summer. In addition, an inversion in the eicosapentaenoic acid (EPA)/arachidonic acid (ARA) and $(n-3)/(n-6)$ ratios was observed in the sheltered site, as a result of the significantly higher levels of ARA and $(n-6)$ fatty acids and lower amounts of EPA and $(n-3)$ fatty acids found in the sheltered limpets. A high variability among patches in the fatty acid composition in the exposed site was found in winter, possibly related to the aggregation of limpets at this time. The differences found between limpets from the exposed and sheltered sites suggest qualitative and quantitative differences in their diets. Additionally, the results show that the spatial aggregation strategy adopted by limpets in sites of great wave and wind exposure does not affect their feeding and reproductive success, at least in the site examined here. In fact, more developed gonads, a higher GSI and an elevated FAME content was found in the exposed population. Possible factors are suggested and discussed to explain these observations.

Keywords: Fatty acid composition; Gastropods; Gonad development; Limpet; *Patella depressa*; Shore exposure; Spatial distribution

1. Introduction

A population of *Patella depressa* Pennant inhabiting an intertidal platform submitted to intense wave and wind exposure was found to aggregate in clumps in rock depressions during winter, in the central coast of Portugal (Cabo Raso). In summer, when the physical conditions are less harsh, limpets assume a random distribution (Boaventura et al., in preparation). This type of aggregation behaviour in the intertidal zone has also been observed in exposed shore populations of dogwhelks *Nucella lapillus* (L.) (Feare, 1970). This species was found to form dense winter aggregations of both adults and immature individuals in clefts and pools, while in the summer months all animals were dispersed in the open shore. Initially it was hypothesised that these aggregations might be related to feeding or protection against predators but it was finally interpreted as a protective behavioural adaptation to water movement (Feare, 1970).

Intra-specific competition for food has been demonstrated to exist and to be an important factor in several grazing gastropod species (Stimson, 1970; Branch, 1975a,b; Underwood, 1976, 1984; Creese and Underwood, 1982; Boaventura et al., 2002). This type of competition is probably more likely to occur and to have more profound consequences in populations submitted to extreme physical conditions and with an aggregated spatial distribution.

The accumulation and depletion of stored reserves in molluscs are dependent on the stage of gonad development, on environmental factors affecting metabolism (mainly temperature) and on the quantity and nutritional value of the food supply (Bayne and Newell, 1983; Gabbott, 1983; Whyte et al., 1990). In spite of some differences in the nature of the reserves, glycogen is normally the major source of energy in molluscs (Barber and Blake, 1981; Voogt, 1983). Lipids, on the other hand, are the nutritive storage product of the gonads (Wenne and Polak, 1989).

A strong correlation between the phase of the reproductive cycle and the lipid content of the gonads has been demonstrated in several bivalve and prosobranch species (Simpson, 1982; Voogt, 1983; Abad et al., 1995; Pazos et al., 1996, 1997). Lipids are primarily stored and subsequently mobilised for gametogenesis, being normally lost during spawning (Barber and Blake, 1981; Gabbott, 1983). In addition, they have also been shown to provide energy for growth during winter conditions when carbohydrate reserves are depleted (Ruiz et al., 1992; Abad et al., 1995; Pazos et al., 1996).

The variation in biochemical composition during a year cycle has been studied in the limpet *Patella vulgata* (Blackmore, 1969a). In this species, there was a marked increase in the polysaccharide content during spring and early summer, coinciding with a time of increasing temperature and algal growth. A peak value was attained at the onset of maturation and this value was maintained in males until spawning, after which it declined steadily. In female limpets, the polysaccharide content dropped as maturation proceeded. The pattern of lipid content variation is similar to that of polysaccharides, although the increase and peak value was seen to occur later. During maturation lipid accumulates in the gonad, while the level in the gonad-free tissue begins to fall (Blackmore, 1969a). No information has been collected on the variation of the biochemical composition in *Patella depressa* but a similar relation between the gonad stage and the lipid content is likely to occur.

Having in mind the relationship between the lipid composition of molluscs and their stage of reproductive development, as well as the quality and quantity of the available diet, the present study was developed with the aim of studying the effect of shore exposure (and associated pattern of spatial distribution) on the feeding and reproductive performance of *P. depressa* populations. In addition, this work presents for the first time a complete description of the fatty acid profile of *P. depressa*.

2. Materials and methods

Two sites, referred to as exposed and sheltered, were chosen in Cabo Raso (Cascais, Portugal) and four patches of 1 m^2 were established within each site. Each site was sampled in the winter (January) and in the summer (August) of 2001. Forty limpets (*P. depressa* Pennant) of the same size class (20–30 mm) were collected in each patch and immediately frozen. The shell length of each animal was measured and the sex and gonad stage were determined, by cutting the foot from its attachment to the visceral mass (Orton et al., 1956; Orton and Southward, 1961). The sexes could be easily assessed by colour, the male gonad being pinkish white or cream and the female brownish or dark green. The gonad stage of development was identified according to the stages defined by Orton et al. (1956) for *P. vulgata*. However, the use of frozen material did not allow determining whether it was in a developing or in a spawning stage.

Twenty of the limpets collected in each patch were used to determine the gonad somatic index (GSI), which was calculated as: GSI = weight of the gonad/weight of the whole soft animal × 100 (modified from Simpson, 1982). For this purpose, the gonad was dissected and weighed and the weight of the body was also recorded after removal from the shell. This

procedure also enabled a preliminary characterization of the gonad development status of the population.

Having in mind that the biochemical composition of aquatic organisms is primarily determined by their diet and physiological status (Bayne and Newell, 1983; Whyte et al., 1990) and that the lipid content of molluscs follows gametogenesis (Blackmore, 1969a; Abad et al., 1995; Pazos et al., 1996), fatty acid analysis of both males and females was performed on a single gonad stage, selected previously as the most abundant in the population (stage II in winter and stage III in summer). Fatty acid analyses were conducted on two females and two males from each patch; their body was removed from the shell and samples were stored in liquid nitrogen for posterior fatty acid analysis. After freeze-drying in a Savant VP100®, samples were ground in a Potter homogenizer with chloroform–methanol–water (2:2:1.8) (Blight and Dyer, 1959). After saponification and esterification of the lipid extracts (Metcalfe and Schmitz, 1961), the fatty acid methyl esters (FAME) were injected into a capillary column (30 m fused silica, 0.32 I.D.) installed in a Varian Star 3400CX gas–liquid chromatograph. Helium was used as carrier gas, at a flow rate of 1 ml/min; oven temperature was 180 °C for 7 min and then 200 °C (with a temperature gradient of 4 °C/min) over a period of 71 min. Both the injector and the FID detector were set at 250 °C. GLC data acquisition and handling was done through a Varian integrator 4290 connected to the GLC. Peak quantification was carried out with a Star Chromatography workstation installed in an IBM PS/1. Peak identification was carried out using as reference well-characterised cod liver oil chromatograms.

The effect of shore exposure on the feeding performance, assessed by fatty acid analyses, was analysed using a three-way mixed model ANOVA. The factors tested were "sex" (fixed, orthogonal, two levels), "sites" (fixed, orthogonal, two levels), "patches" (random, nested within sites, four levels). Cochran's *C*-test was used to check homogeneity of variance. Where this assumption was violated, logarithmic transformations were used (Underwood, 1997). For each fatty acid group, data were analysed at two dates—once in the winter period, which corresponded to the period of aggregated distribution in the exposed site, and once during summer, where limpets were apparently randomly distributed at both sites. Only the effects of sex and exposure were tested at each one of these dates. Tests of homogeneity, ANOVA and SNK (Student–Newman–Keuls) a posteriori comparison tests were done using GMAV5 for Windows Statistical Software (Institute of Marine Ecology, Sydney, Australia).

3. Results

3.1. Sex and gonad development

The maximum, minimum and average shell length, as well as the proportion of males, females and neuters recorded in the winter and summer populations of both sites can be seen in Table 1. The results show that individuals from the same size class were examined, which renders the results comparable. In addition, no difference was noted in shell length between males and females (25.1 ± 1.82 and 25.0 ± 1.62, respectively). As for the sex

Table 1
Maximum, minimum and average values of shell length, and proportion of males, females and neuters in the winter and summer populations of the exposed and sheltered sites ($n = 160$)

	Winter		Summer	
	Exposed	Sheltered	Exposed	Sheltered
Shell length (mm)				
Maximum	29.2	29.9	30.1	29.7
Minimum	20.3	21.5	20.3	21.5
Average (± S.D.)	24.8 ± 1.81	25.4 ± 1.67	25.1 ± 1.71	25.2 ± 1.72
Sex ratio				
Males (%)	63.8	58.5	63.6	52.5
Females (%)	33.7	37.7	36.4	46.9
Neuters (%)	2.5	3.8	0.0	0.6

ratio, males were found to be slightly preponderant in both sites, in winter and summer. The proportion of neuters was much higher during the winter period.

Results from the distribution of gonad development stages in the limpet population of the exposed and sheltered sites, both in winter and summer periods, revealed a higher dispersion of gonad stages and more advanced stages of development in the exposed site (Figs. 1 and 2). In winter, *P. depressa* presents a gonad development from stage I to stage III in the exposed site, while the maximum gonad stage observed in the sheltered site was stage II (Fig. 1). In summer there was a higher development of the gonads in both sites, comparatively to the winter situation, but in the exposed site gonads in stage V were already present, whereas in the sheltered site the most advanced limpets were still in stage IV (Fig. 2). In addition, the bulk of the population was found in a more advanced stage of gonad development in the exposed site-in winter, the majority of the male and female population (56% and 66%, respectively) was found in stage II, whereas in the sheltered site the gonads of most limpets (56% and 57% of the male and female population, respectively) were still in stage I (Fig. 1). In summer, the same trend was observed, although not so pronounced, with most of the exposed population being either in stage III and IV (42% of males in stage IV, and 36% and 34% of females in stages III and IV,

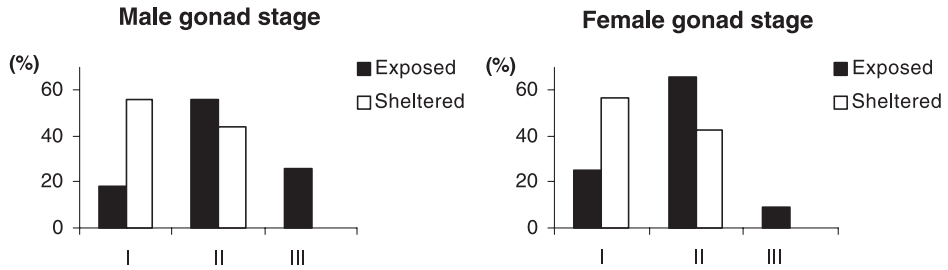

Fig. 1. Distribution of gonad development stages in the male and female limpet population of the exposed and sheltered site, in winter.

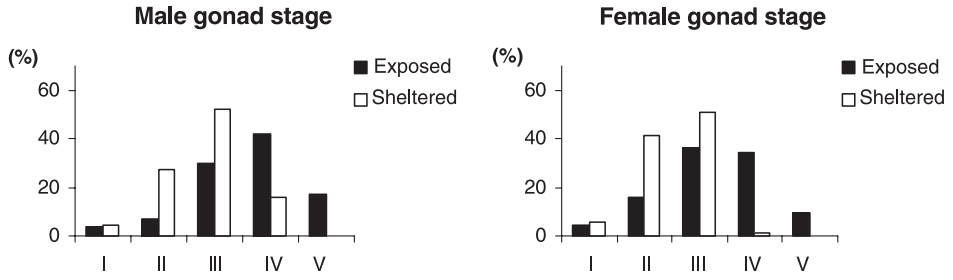

Fig. 2. Distribution of gonad development stages in the male and female limpet population of the exposed and sheltered site, in summer.

respectively), while 52% of the males and 51% of the females in the sheltered population had their gonads in stage III (Fig. 2).

Some difficulties were encountered in the determination of the gonad somatic index (GSI), particularly during gonad removal. The individuals had to be dissected partially frozen, to avoid the loss of gonad tissue and fluids that occurred when gonads were completely thawed. Therefore, a precise measurement of the gonad weight was difficult to obtain, which accounts for the high standard deviation values associated with the GSI determination (Table 2). Nonetheless, some general observations can still be made. As expected, an increase in the GSI in more advanced stages of gonad development was recorded, as a result of the increment in gonad weight during development. The males show a higher gonad weight relative to their body weight than the females, in spite of their apparently comparable volume. An increase in the average male and female GSI was observed from winter to summer, as a consequence of the more advanced stage of

Table 2
Gonad somatic index (± S.D.) of each male and female gonad stage of development in the exposed and sheltered sites, during winter and summer ($n = 80$ per site)

	I	II	III	IV	V	Average
Winter						
Exposed						
Male	8.6 ± 3.59	9.5 ± 2.95	12.7 ± 3.28	–	–	10.5 ± 3.58
Female	4.0 ± 2.12	4.6 ± 2.00	5.3 ± 3.46	–	–	4.6 ± 2.25
Sheltered						
Male	5.5 ± 1.79	6.4 ± 2.23	–	–	–	5.8 ± 1.96
Female	3.3 ± 1.54	4.8 ± 1.34	–	–	–	3.7 ± 1.62
Summer						
Exposed						
Male	10.0 ± 3.66	11.8 ± 2.77	15.7 ± 3.48	19.9 ± 4.44	25.6 ± 2.55	17.1 ± 5.61
Female	3.5 ± 0.81	7.2 ± 2.81	11.6 ± 4.30	12.9 ± 4.07	17.5 ± 0.00	10.2 ± 4.79
Sheltered						
Male	4.6 ± 3.26	7.2 ± 2.01	9.8 ± 2.62	10.4 ± 2.51	–	8.4 ± 2.90
Female	3.4 ± 1.36	5.3 ± 2.65	7.1 ± 1.83	6.6 ± 0.00	–	5.9 ± 2.46

development of the limpet population in summer. Additionally, a higher average GSI was determined in the exposed population both in winter and summer, again as a result of the greater gonad development in this site. A curious result was obtained when comparing the GSI of the same stage of gonad development in males and females from the exposed and sheltered sites. Limpets from the exposed site, particularly the males, presented a higher GSI than the corresponding stage in the sheltered site. This was observed both in the winter and summer periods.

3.2. Fatty acid composition

The analysis of the fatty acid composition of male and female limpets in the exposed and sheltered sites revealed no important qualitative differences in the limpet fatty acid profile, both in winter and summer (Table 3). However, some differences were observed in the quantities in which the major fatty acids were present. The quantitatively most important fatty acids were the saturated fatty acids (SFA) 16:0, 14:0 and 18:0, the monounsaturated fatty acids (MUFA) 18:1($n-7$), 18:1($n-9$), 16:1($n-7$) and 20:1($n-9$) and the polyunsaturated fatty acids (PUFA) 20:5($n-3$) (EPA; eicosapentaenoic acid) and 20:4($n-6$) (ARA; arachidonic acid).

The biochemical data was analysed through analyses of variance in order to determine whether significant differences existed in the level of total fatty acid methyl esters (FAME), of the main classes of fatty acids (SFA, MUFA, PUFA, HUFA, total ($n-3$) and ($n-6$) series), as well as of the most abundant PUFA (EPA and ARA). The results of the statistical analysis conducted for the winter samples revealed significant differences among patches for FAME, HUFA, ($n-3$), ($n-6$), PUFA, ARA, EPA (Table 4). SNK tests revealed the existence of a general higher variability and some significant differences among patches at the exposed site but no significant differences on the fatty acid content were noted between the various patches in the sheltered site. An interaction between sex and patch was found for SFA and MUFA (Table 4). However, females had a significantly higher amount of these fatty acid classes in most patches of the exposed site, while no significant differences were found between sexes in the sheltered site (Table 4, SNK tests). The total FAME content was also significantly higher in females than males, although these results should be regarded with some caution in summer, when variances were heterogeneous. The same statistical analysis was conducted in summer and, in this case, the amount of fatty acids was significantly higher in the exposed than in the sheltered site for most of the analysed classes, with the exception of ARA and total ($n-6$) fatty acids, where the inverse relation occurred. Significant differences between males and females were found in summer for FAME, SFA and MUFA (Table 5). Female limpets presented a significantly higher content of total FAME than the males in the same population. This difference is due to the higher content of SFA and MUFA found in the female, given that the PUFA absolute level was not significantly different from the male body.

An interesting result was the presence of very low amounts (or even complete absence, in the sheltered site in winter) of 22:6($n-3$) (DHA; docosahexaenoic acid), in both male and female limpets (Table 3). This fatty acid is usually quite abundant in the marine environment, being considered an essential fatty acid. It should also be noted that,

Table 3
Fatty acid composition (μg/mg of dry weight) of the male and female *P. depressa* soft body, in the exposed and sheltered sites, during winter and summer ($n = 8$ per sex per site)

Fatty acids μg/mg DW	Winter				Summer			
	Exposed		Sheltered		Exposed		Sheltered	
	Male	Female	Male	Female	Male	Female	Male	Female
13:0	0.06 ± 0.02	0.05 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.10 ± 0.03	0.10 ± 0.01	0.04 ± 0.03	0.07 ± 0.02
14:0	1.58 ± 0.83	2.52 ± 1.28	0.48 ± 0.06	1.11 ± 0.26	2.15 ± 1.02	3.88 ± 0.45	1.20 ± 0.35	2.46 ± 0.25
15:0	0.15 ± 0.03	0.16 ± 0.03	0.15 ± 0.03	0.16 ± 0.02	0.39 ± 0.13	0.51 ± 0.08	0.27 ± 0.06	0.36 ± 0.04
16:0	2.64 ± 0.82	5.14 ± 2.21	1.62 ± 0.26	2.88 ± 0.49	5.59 ± 2.21	9.90 ± 0.56	3.49 ± 0.78	6.79 ± 0.78
17:0	0.13 ± 0.03	0.14 ± 0.03	0.13 ± 0.02	0.15 ± 0.02	0.25 ± 0.07	0.34 ± 0.04	0.17 ± 0.02	0.23 ± 0.03
18:0	1.28 ± 0.35	1.40 ± 0.52	0.87 ± 0.14	0.97 ± 0.08	1.76 ± 0.42	2.11 ± 0.24	1.18 ± 0.08	1.62 ± 0.15
20:0	0.22 ± 0.05	0.23 ± 0.09	0.19 ± 0.04	0.19 ± 0.04	0.03 ± 0.04	0.07 ± 0.05	0.00 ± 0.00	0.06 ± 0.07
ΣSFA	6.05 ± 2.06	9.67 ± 4.17	3.45 ± 0.50	5.47 ± 0.79	10.27 ± 3.85	16.92 ± 1.16	6.34 ± 1.18	11.58 ± 1.17
16:1($n-9$)	0.16 ± 0.09	0.00 ± 0.00	0.09 ± 0.05	0.00 ± 0.00	0.05 ± 0.06	0.00 ± 0.00	0.03 ± 0.03	0.00 ± 0.00
16:1($n-7$)	1.10 ± 0.59	1.46 ± 0.69	0.31 ± 0.05	0.60 ± 0.15	1.25 ± 0.60	1.94 ± 0.19	0.74 ± 0.24	1.28 ± 0.16
17:1($n-8$)	0.03 ± 0.02	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.06 ± 0.05	0.05 ± 0.04	0.09 ± 0.07	0.12 ± 0.05
18:1($n-9$)	0.62 ± 0.18	1.55 ± 0.56	0.46 ± 0.09	1.01 ± 0.24	1.26 ± 0.49	2.94 ± 0.56	0.90 ± 0.17	2.33 ± 0.41
18:1($n-7$)	2.59 ± 1.00	3.64 ± 1.67	1.11 ± 0.18	1.76 ± 0.31	4.04 ± 1.12	5.89 ± 0.62	2.34 ± 0.43	3.77 ± 0.48
18:1($n-5$)	0.04 ± 0.01	0.06 ± 0.02	0.06 ± 0.03	0.04 ± 0.02	0.01 ± 0.01	0.00 ± 0.00	0.05 ± 0.02	0.01 ± 0.02
20:1($n-9$)	0.88 ± 0.31	1.39 ± 0.50	0.56 ± 0.25	0.95 ± 0.12	1.24 ± 0.30	2.17 ± 0.32	1.01 ± 0.08	1.66 ± 0.15
20:1($n-7$)	0.43 ± 0.13	0.71 ± 0.32	0.19 ± 0.10	0.36 ± 0.08	0.63 ± 0.16	1.11 ± 0.13	0.42 ± 0.09	0.74 ± 0.10
20:1($n-5$)	0.12 ± 0.06	0.21 ± 0.12	0.08 ± 0.03	0.11 ± 0.03	0.22 ± 0.05	0.37 ± 0.04	0.16 ± 0.06	0.25 ± 0.03
22:1($n-11$)	0.07 ± 0.09	0.10 ± 0.05	0.00 ± 0.00	0.06 ± 0.06	0.04 ± 0.04	0.11 ± 0.09	0.00 ± 0.00	0.03 ± 0.03
22:1($n-7$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.03	0.18 ± 0.06	0.04 ± 0.05	0.16 ± 0.09
ΣMUFA	6.04 ± 1.99	9.19 ± 3.91	2.86 ± 0.52	4.94 ± 0.97	8.87 ± 2.54	14.81 ± 0.80	5.82 ± 0.97	10.40 ± 1.01
Iso 15:0	0.05 ± 0.02	0.03 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.11 ± 0.03	0.12 ± 0.04	0.06 ± 0.03	0.07 ± 0.02
Anteiso 15:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.02 ± 0.03	0.00 ± 0.00	0.05 ± 0.02
Iso 16:0	0.12 ± 0.05	0.11 ± 0.03	0.10 ± 0.02	0.14 ± 0.03	0.30 ± 0.09	0.34 ± 0.06	0.25 ± 0.10	0.40 ± 0.10

Anteiso 16:0	0.06 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	0.08 ± 0.04	0.05 ± 0.02	0.04 ± 0.02	0.05 ± 0.01
Iso 17:0	0.15 ± 0.06	0.26 ± 0.10	0.11 ± 0.03	0.18 ± 0.05	0.44 ± 0.12	0.67 ± 0.08	0.24 ± 0.05	0.41 ± 0.05
Anteiso 17:0	0.04 ± 0.02	0.07 ± 0.03	0.06 ± 0.02	0.09 ± 0.02	0.13 ± 0.03	0.21 ± 0.03	0.14 ± 0.03	0.23 ± 0.04
ΣBranched	0.40 ± 0.13	0.50 ± 0.14	0.37 ± 0.07	0.50 ± 0.09	1.07 ± 0.28	1.41 ± 0.19	0.74 ± 0.18	1.20 ± 0.18
16:2($n-4$)	0.20 ± 0.10	0.20 ± 0.09	0.09 ± 0.02	0.12 ± 0.02	0.47 ± 0.22	0.47 ± 0.16	0.40 ± 0.21	0.49 ± 0.17
16:3($n-4$)	0.82 ± 0.24	0.65 ± 0.29	0.70 ± 0.21	0.64 ± 0.23	0.72 ± 0.22	0.59 ± 0.31	0.51 ± 0.15	0.32 ± 0.25
16:3($n-3$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.23 ± 0.10	0.31 ± 0.06	0.10 ± 0.03	0.16 ± 0.03
16:4($n-3$)	0.09 ± 0.02	0.08 ± 0.02	0.08 ± 0.01	0.09 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16:4($n-1$)	0.13 ± 0.13	0.20 ± 0.13	0.08 ± 0.10	0.11 ± 0.09	0.24 ± 0.15	0.40 ± 0.10	0.32 ± 0.12	0.36 ± 0.17
18:2($n-6$)	0.11 ± 0.04	0.14 ± 0.04	0.11 ± 0.05	0.16 ± 0.06	0.33 ± 0.11	0.42 ± 0.10	0.51 ± 0.23	0.66 ± 0.23
18:3($n-3$)	0.26 ± 0.05	0.27 ± 0.05	0.23 ± 0.04	0.30 ± 0.04	1.27 ± 0.46	1.70 ± 0.43	0.86 ± 0.37	1.09 ± 0.16
18:4($n-3$)	0.59 ± 0.19	0.48 ± 0.23	0.40 ± 0.12	0.35 ± 0.12	0.41 ± 0.16	0.55 ± 0.18	0.22 ± 0.08	0.14 ± 0.08
20:2($n-6$)	0.30 ± 0.08	0.40 ± 0.17	0.31 ± 0.05	0.36 ± 0.07	0.32 ± 0.11	0.48 ± 0.06	0.37 ± 0.07	0.55 ± 0.12
20:3($n-6$)	0.10 ± 0.04	0.21 ± 0.16	0.09 ± 0.02	0.15 ± 0.03	0.21 ± 0.08	0.32 ± 0.05	0.19 ± 0.08	0.34 ± 0.07
20:4($n-6$)	2.14 ± 0.49	2.01 ± 0.51	2.37 ± 0.36	2.43 ± 0.41	1.60 ± 0.26	1.83 ± 0.22	2.64 ± 0.33	2.75 ± 0.51
20:3($n-3$)	0.40 ± 0.09	0.37 ± 0.08	0.36 ± 0.04	0.44 ± 0.12	0.69 ± 0.22	0.99 ± 0.24	0.46 ± 0.12	0.63 ± 0.12
20:4($n-3$)	0.16 ± 0.08	0.19 ± 0.09	0.04 ± 0.02	0.09 ± 0.04	0.35 ± 0.18	0.59 ± 0.16	0.14 ± 0.09	0.29 ± 0.07
20:5($n-3$)	4.51 ± 2.35	3.97 ± 2.18	1.50 ± 0.26	1.43 ± 0.32	4.31 ± 1.06	3.67 ± 0.47	2.30 ± 0.46	1.99 ± 0.51
21:5($n-3$)	0.10 ± 0.05	0.11 ± 0.05	0.04 ± 0.01	0.05 ± 0.03	0.06 ± 0.06	0.08 ± 0.05	0.10 ± 0.08	0.11 ± 0.05
22:4($n-6$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.05 ± 0.02	0.01 ± 0.01	0.01 ± 0.01
22:5($n-3$)	0.18 ± 0.11	0.22 ± 0.14	0.04 ± 0.02	0.05 ± 0.03	0.25 ± 0.09	0.33 ± 0.07	0.10 ± 0.03	0.12 ± 0.05
22:6($n-3$)	0.14 ± 0.12	0.19 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.08	0.13 ± 0.06	0.02 ± 0.03	0.04 ± 0.03
24:1($n-9$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.03	0.05 ± 0.08	0.03 ± 0.07	0.01 ± 0.02
ΣPUFA	10.03 ± 3.08	9.47 ± 3.83	6.33 ± 0.94	6.63 ± 1.25	11.08 ± 2.61	12.42 ± 1.81	8.82 ± 0.94	9.55 ± 1.35
ΣHUFA	8.02 ± 2.69	7.66 ± 3.24	4.74 ± 0.71	4.98 ± 0.94	7.89 ± 1.86	8.45 ± 1.16	6.30 ± 0.66	6.83 ± 1.13
Σ($n-3$)	6.43 ± 2.88	5.87 ± 2.85	2.68 ± 0.40	2.79 ± 0.64	7.66 ± 2.10	8.33 ± 1.26	4.28 ± 0.77	4.57 ± 0.85
Σ($n-6$)	2.64 ± 0.63	2.75 ± 0.85	2.87 ± 0.44	3.09 ± 0.49	2.46 ± 0.50	3.10 ± 0.41	3.71 ± 0.51	4.30 ± 0.77
($n-3$)/($n-6$)	2.58 ± 1.17	2.11 ± 0.75	0.93 ± 0.05	0.90 ± 0.11	3.11 ± 0.60	2.69 ± 0.24	1.17 ± 0.28	1.08 ± 0.23
EPA/ARA	2.26 ± 1.22	1.94 ± 0.85	0.63 ± 0.05	0.59 ± 0.08	2.71 ± 0.58	2.02 ± 0.22	0.89 ± 0.23	0.74 ± 0.18
ΣTotal FAME	22.52 ± 7.07	28.81 ± 11.79	13.01 ± 1.96	17.54 ± 2.33	31.29 ± 7.81	45.56 ± 3.34	21.71 ± 2.97	32.73 ± 2.98

Table 4
Analysis of variance on the fatty acid composition (μg/mg of dry weight) of *P. depressa* soft body for the samples collected in winter

Source of variation	*df*	SFA		MUFA		PUFA		HUFA		FAME		(*n* − 3)		(*n* − 6)		EPA		ARA	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Sex = Se	1	63.20	17.48**	54.76	18.57**	0.14	0.07 ns	0.03	0.02 ns	233.77	10.53*	0.41	0.46 ns	0.21	0.65 ns	0.74	1.34 ns	0.01	0.06 ns
Site = Si	1	91.90	4.84 ns	110.48	6.08*	85.28	3.68 ns	70.89	4.20 ns	863.93	4.81 ns	93.40	5.89 ns	0.67	0.78 ns	61.61	6.27*	0.85	1.98 ns
Patch (Site) = Pa(Si)	6	19.00	14.56***	18.17	17.59***	23.17	10.63***	16.88	11.21***	179.64	16.42***	15.86	13.55***	0.85	3.60*	9.83	14.45***	0.43	3.34*
Se × Si	1	5.00	1.38 ns	2.28	0.77 ns	1.45	0.72 ns	0.74	0.52 ns	6.20	0.28 ns	0.91	1.02 ns	0.02	0.07 ns	0.46	0.83 ns	0.07	0.40 ns
Se × Pa(Si)	6	3.62	2.77*	2.95	2.85*	2.02	0.93 ns	1.43	0.95 ns	22.20	2.03 ns	0.89	0.76 ns	0.32	1.37 ns	0.55	0.81 ns	0.17	1.30 ns
Res	16	1.31		1.03		2.18		1.51		10.94		1.17		0.24		0.68		0.13	
Cochran's test		*C* = 0.19 ns		*C* = 0.19 ns		*C* = 0.29 ns		*C* = 0.37 ns		*C* = 0.23 ns		*C* = 0.39 ns		*C* = 0.27 ns		*C* = 0.43 ns		*C* = 0.36 ns	
Transformation		(none)		(none)		(none)		(none)		(none)		(none)		(none)		(none)		(none)	
SNK tests		Se × Pa(Si), S.E. = 0.81 Exposed P1, M < F** P2, M < F** P3, M < F** P4, M = F ns Sheltered P1, M = F ns P2, M = F ns P3, M = F ns P4, M = F ns		Se × Pa(Si), S.E. = 0.72 Exposed P1, M < F** P2, M < F** P3, M < F* P4, M = F ns Sheltered P1, M < F** P2, M = F ns P3, M = F ns P4, M = F ns		Patch(Si), S.E. = 0.73 Exposed P1>P2 = P3>P4* Sheltered P1 = P2 = P3 = P4 ns		Patch(Si), S.E. = 0.61 Exposed P1>P2 = P3>P4* Sheltered P1 = P2 = P3 = P4 ns		Sex, S.E. = 1.18 M < F* Patch(Si), S.E. = 1.65 Exposed P1>P2 = P3>P4** Sheltered P1 = P2 = P3 = P4 ns		Patch(Si), S.E. = 0.54 Exposed P1>P2 = P3>P4** Sheltered P1 = P2 = P3 = P4 ns		Patch(Si), S.E. = 1.65 Exposed No logical sequence* Sheltered P1 = P2 = P3 = P4 ns		Patch(Si), S.E. = 0.41 Exposed No logical sequence* Sheltered P1 = P2 = P3 = P4 ns		Patch(Si), S.E. = 0.18 Exposed No logical sequence* Sheltered P1 = P2 = P3 = P4 ns	

ns = not significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 5
Analysis of variance on the fatty acid composition (μg/mg of dry weight) of *P. depressa* soft body for the samples collected in summer

Source of variation	*df*	SFA		MUFA		PUFA		HUFA		FAME		(*n* − 3)		(*n* − 6)		EPA		ARA	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Sex = Se	1	282.68	70.81***	221.50	94.39***	8.57	1.74 ns	2.37	1.16 ns	1278.79	41.18***	0.06	1.17 ns	2.99	4.68 ns	1.80	5.91 ns	0.23	1.16 ns
Site = Si	1	171.82	55.63***	111.42	116.24***	52.69	94.14***	20.61	40.75***	1003.63	227.68***	2.74	354.82***	12.08	106.25***	27.14	87.90***	7.70	50.51***
Patch (Site) = Pa(Si)	6	3.09	0.55 ns	0.96	0.35 ns	0.56	0.16 ns	0.51	0.27 ns	4.41	0.17 ns	0.01	0.16 ns	0.12	0.42 ns	0.31	0.54 ns	0.15	1.97 ns
Se × Si	1	3.97	0.99 ns	3.69	1.57 ns	0.74	0.15 ns	0.00	0.00 ns	21.17	0.68 ns	0.01	0.06 ns	0.00	0.01 ns	0.22	0.73 ns	0.03	0.14 ns
Se × Pa(Si)	6	3.99	0.71 ns	2.35	0.86 ns	4.93	1.40 ns	2.03	1.08 ns	31.05	1.19 ns	0.05	1.08 ns	0.64	2.36 ns	0.30	0.54 ns	0.20	2.62 ns
Res	16	5.64		2.72		3.53		1.89		26.04		0.05		0.27		0.57		0.08	
Cochran's test		*C* = 0.46 ns		*C* = 0.44 ns		*C* = 0.41 ns		*C* = 0.29		*C* = 0.56		*C* = 0.37 ns		*C* = 0.30 ns		*C* = 0.33 ns		*C* = 0.33	
Transformation		(none)		(none)		(none)		(none)		$p < 0.05$		(Ln(x))		(none)		(none)		(none)	
SNK tests		Sex, S.E. = 0.50 M < F**		Sex, S.E. = 0.38 M < F**						Sex, S.E. = 1.39 M < F**									
		Site,S.E. = 0.44 E>S**		Site,S.E. = 0.24 E>S**		Site,S.E. = 0.19 E>S**		Site,S.E. = 0.18 E>S**		Site,S.E. = 0.52 E>S**		Site,S.E. = 0.02 E>S**		Site,S.E. = 0.08 E < S**		Site,S.E. = 0.14 E>S**		Site,S.E. = 0.10 E < S**	

ns = not significant.
** $p < 0.01$.
*** $p < 0.001$.

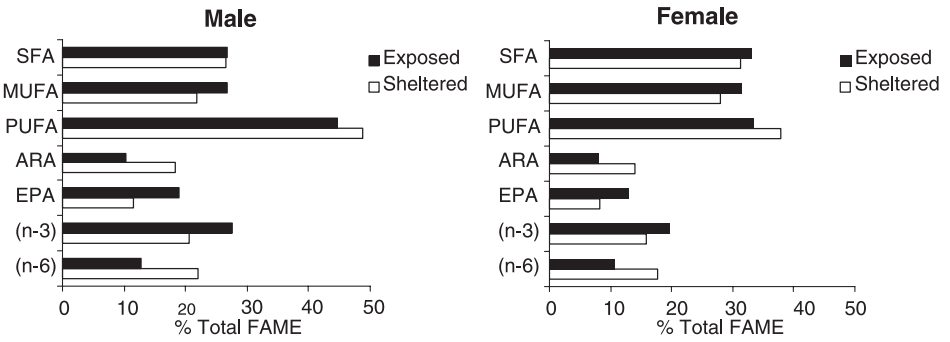

Fig. 3. Relative composition (% of total FAME) of selected classes of fatty acids in the male and female *P. depressa* soft body, in the exposed and sheltered sites, in winter.

independently of site and season, females always presented slightly higher absolute amounts of DHA than males.

To eliminate quantitative differences between the analysed samples and better visualise differences in the proportion of the different fatty acids, the fatty acid composition was also expressed in relative terms, as a percentage of total fatty acids (Figs. 3 and 4). The results reveal that limpets in the exposed site present a similar or slightly higher percentage of SFA and MUFA than the corresponding sex in the sheltered site. On the contrary, male and female limpets in the sheltered site have a higher percentage of PUFA (especially ARA) than the corresponding sex on the exposed site. However, there were exceptions to this rule and some PUFA were present in higher proportions in the exposed site, EPA being the main exception. Males are characterised by presenting higher PUFA and HUFA percentages (particularly 18:3($n-3$), 18:4($n-3$), ARA and EPA). Female limpets, on the other hand, have a higher proportion of SFA (14: and 16:0) and MUFA (16:1($n-7$), 18:1($n-9$), 18:1($n-7$) and 20:1($n-9$)) than males.

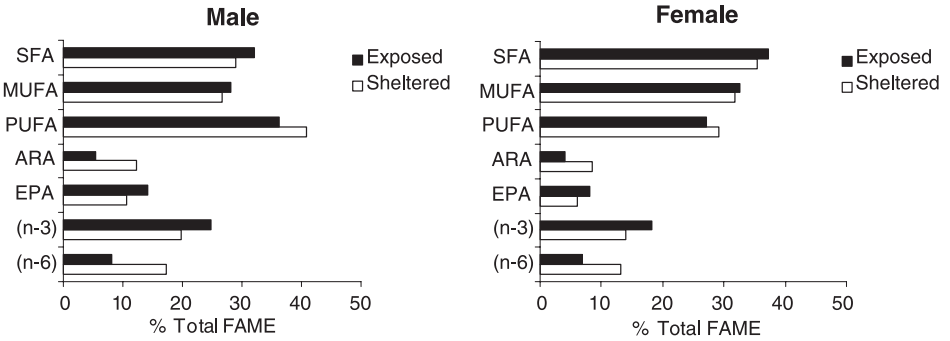

Fig. 4. Relative composition (% of total FAME) of selected classes of fatty acids in the male and female *P. depressa* soft body, in the exposed and sheltered sites, in summer.

4. Discussion

Few studies on the biochemical composition of marine molluscs have investigated the effects of ecological factors (e.g. Ruiz et al., 1992; Pazos et al., 1997). The experimental design and methodology used in this study tested the effect of shore exposure and spatial distribution patterns on the fatty acid composition of male and female limpets. The major constraint to this approach was the reduced number of replicates due to limits imposed by the cost and time required for each biochemical analysis. Even so, we were able to obtain interesting results regarding the quantitatively most important fatty acids in the species *P. depressa* and analyse the influence of different ecological conditions. Additionally, the complete fatty acid profile of *P. depressa* (male and female) was described for the first time in the present work (Table 3).

4.1. Sex and gonad development

P. depressa is a summer breeding species in south–west England; gonad development begins in April–May, as temperature rises, and spawning occurs between the latter half of July and early September (Orton and Southward, 1961). In the central coast of Portugal, spawning seems to occur later, in October and November (Guerra and Gaudêncio, 1986).

The sex ratio was found to be relatively constant in both populations and at different times of the year (58.5–63.8% males:33.7–37.7% females), except for the relatively more even proportion of males and females in the sheltered site during summer (52.5% and 46.9%, respectively). The sex ratio in the size class of 20–30 mm of *P. depressa* appears therefore to be independent of patterns of spatial distribution, season and physical conditions. Orton and Southward (1961), when studying a *P. depressa* population from the North Cornish coast, found that males reached a maximum proportion of about two-thirds of the 25–35 mm population, while females consisted of the remaining one third. These results are consistent with the present study.

The examination of the gonad stage of development in both populations during winter and summer revealed that male and female limpets in the exposed site are characterised by a greater range of stages and by a higher degree of gonad development. Moreover, the percentage of neuters was lower in the exposed site than in the sheltered site. In winter most of the limpets in the sheltered site were in stage I (with gonad development going up to stage II), while in the exposed site the bulk of the population was found in stage II (with the most advanced limpets being in stage III). In summer there was a higher dispersion of gonad stages, with individuals being found from stage I up to stage IV (in the sheltered site) or stage V (in the exposed site). However, stages III and IV were preponderant in the exposed site, while stage III prevailed in male and female limpets in the sheltered site.

The high degree of gonad stage dispersion found in the present study, particularly as the reproductive cycle progresses, is typical of *Patella* species that possess a long reproductive cycle, with an extended spawning period, in which the various stages are prolonged and tend to overlap. In *P. vulgata*, for instance, it may take six or more months from the start of spawning until the whole population reaches the resting or developing stages (Orton et al., 1956).

The mean size of the gonad has been found to be greater in larger limpets than in smaller animals, particularly at the time of maturity (Blackmore, 1969b). However, in the present work, the size class was fixed and did not vary between sites and sex. Curiously, male and female limpets were found to have a higher GSI in the exposed site, both in winter and summer. This could either be explained by an increased gonad weight or by a lower body weight of the limpets in this site. However, the second possibility does not seem likely since, if there was a difference in body weight between limpets in both sites, one would expect that limpets in the exposed site would develop a more voluminous body, to cope with the harsher physical environment experienced in this site. On the other hand, males were shown to have a higher GSI than the corresponding female stage, independently of the site from which they were collected. This substantiates the results obtained by Blackmore (1969b) in *P. vulgata*, in which females from the 15–25 and >30 mm size group tended to have smaller gonads than the males. In addition, males from the sub-Antarctic limpet *Nacella (Patinigera) macquariensis* were also found to have significantly larger gonad indices than females (Simpson, 1982). Nevertheless, the higher gonad weight found in males cannot be attributed to a higher lipid content but must be due to some other component (see fatty acid analysis data).

4.2. Fatty acid composition

Most of the lipid reserves of molluscs are usually found in the gonads, particularly at more advanced stages of development (Simpson, 1982; Gabbott, 1983; Voogt, 1983; Abad et al., 1995; Pazos et al., 1996).

The level of total fatty acids measured in the whole body of *P. depressa* was not very high and there might be several explanations for this fact. Firstly, lipids are not the main energy reserve in molluscs, which are generally characterised by low total fat contents (Bonnet et al., 1974). Secondly, the results are expressed in terms of the amount of total fatty acids in relation to total body weight and there are tissues and organs which are a substantial part of the dry weight of the animal that do not provide any lipids. Finally, the limpets that were analysed in both winter and summer were at a relatively early stage of gonad development (stage II and stage III, respectively). Being the gonad the major organ in which lipids can be found, a high total FAME content is not foreseen when this organ is only poorly developed.

Not much work has been conducted on the fatty acid composition of prosobranch gastropods. Voogt (1983) cites a few studies performed on some *Haliotis* species and on *Chlorostoma argyrostoma*, in which the main fatty acids were shown to be, in order of decreasing abundance, 16:0, 18:1, 20:4, 20:5 and 22:5. The same fatty acids were found to predominate in *Haliotis laevigata* and *Haliotis rubra* (Dunstan et al., 1996). Much of the work that has been done on the fatty acid composition of molluscs has focused on bivalves, most probably as a result of their great commercial importance and impact on public health (Abad et al., 1995; Pazos et al., 1996). Given the completely different feeding habits and particular environmental conditions to which limpets are exposed, a comparison with the fatty acid profile of bivalve species must be conducted with care.

The fatty acid analysis of the *P. depressa* tissues revealed that the quantitatively most important SFA are 16:0, 14:0, and 18:0, the predominant MUFA are 18:1($n-7$),

18:1($n-9$), 16:1($n-7$) and 20:1($n-9$), while the main PUFA were found to be EPA and ARA. The results obtained in the present study show that the limpet fatty acid profile conforms with the common trend which is characteristic of molluscs and marine animals in general. The predominant SFA and MUFA were generally the same as in most mollusc species, although the abundance of each fatty acid in different species may vary considerably (Gardner and Riley, 1972; Bonnet et al., 1974; Pastoriza et al., 1981; Wenne and Polak, 1989; Abad et al., 1995; Dunstan et al., 1996; Pazos et al., 1996, 1997). A high EPA content has been found in many marine molluscs, such as *Ostrea edulis*, *Crassostrea gigas*, *Crassostrea virginica*, *Mercenaria mercenaria*, *Mya arenaria*, *Spisula solidissima*, *Chlamys opercularis*, *Crepidula fornicata*, *Mytilus edulis*, *Neptunea antiqua*, *Macoma balthica*, *Argopecten gibbus*, *Placopecten magellanicus*, *Pecten maximus*, *H. laevigata*, *H. rubra* and *P. vulgata* (Shimma and Taguchi, 1964; Gardner and Riley, 1972; Bonnet et al., 1974; Wenne and Polak, 1989; Abad et al., 1995; Dunstan et al., 1996; Pazos et al., 1996, 1997). A high ARA level has been reported in some prosobranch gastropods and, in certain cases, it has been shown that linoleic acid (18:2($n-6$)) can be rapidly synthesised from acetate and converted into ARA, which may therefore accumulate in considerable amounts (Voogt, 1983; Dunstan et al., 1996). On the other hand, the low DHA level observed in *P. depressa* is unusual for marine animals, including marine molluscs, given that it is normally one of the most abundant fatty acids in marine lipids (Bonnet et al., 1974; Wenne and Polak, 1989; Abad et al., 1995; Pazos et al., 1996, 1997). Nevertheless, low or null DHA contents have also been reported in other molluscs and prosobranch gastropods (Shimma and Taguchi, 1964; Gardner and Riley, 1972; Pastoriza et al., 1981; Voogt, 1983; Dunstan et al., 1996).

An inversion in the EPA/ARA and ($n-3$)/($n-6$) ratios was observed in the sheltered site. In summer, limpets from the exposed site showed significant higher amounts of EPA and fatty acids from the ($n-3$) series, as well as lower levels of ARA and, consequently, of fatty acids from the ($n-6$) series. The limpets in the exposed site had also higher relative levels of longer chain PUFA, such as 22:5($n-3$) and DHA. The composition of PUFA in the analysed species can only be related to their diet, given that marine molluscs cannot synthesise PUFA de novo (Abad et al., 1995). Therefore, the differences found between limpets in the exposed and sheltered sites suggest qualitative differences in their diets.

The physical environment experienced by the organisms inhabiting the intertidal zone will undoubtedly affect the community structure, influencing differently the recruitment, survival and growth of different species of algae. It is not strange therefore that the macrophyte and microalgal community (diatoms, algal spores and detrital deposits) will be composed of different species in the exposed and in the sheltered sites, resulting in differences in the diet of the grazing populations inhabiting these sites. For instance, foliose algae were found to grow at the lowest levels of the shores, being more abundant where the wave action is greater (Underwood and Jernakoff, 1984). In addition, a greater exposure to wave shock has been shown to be responsible for an extended distribution of algae until higher levels (Underwood and Jernakoff, 1984).

Several species of marine algae have been studied and it has been established that the total lipids of different classes have relatively characteristic fatty acid compositions. The existence of so called "marker" fatty acids has allowed researchers to make assumptions

regarding the microalgal diet of different marine animals. For instance, it has been determined that diatoms (Bacillariophyceae) tend to be rich in EPA and to a lesser extent in C16 PUFA, while dinoflagellates (Dinophyceae) are richer in DHA. Green algae (Chlorophyceae), Cryptophaceae and Haptophyceae contain low proportions of C20 PUFA but are normally rich in C18 PUFA (especially 18:4($n-3$)), whereas red algae (Rhodophyta), besides being rich in ($n-3$) PUFA, may present considerable amounts of ARA. The main C20 PUFA in brown macroalgae are ARA and EPA, but they also contain significant levels of C18 ($n-3$) PUFA (Chuecas and Riley, 1969; Sargent et al., 1989; Dunstan et al., 1996). As already mentioned, the EPA and DHA levels were found to be greater in limpets in the exposed site, while ARA was found at a much higher relative level in the limpets from the sheltered site. The C18 PUFA (characteristic of green algae) 18:3($n-3$) and 18:4($n-3$) were found at relatively similar levels in both sites. Taking into account the fatty acid composition typical of each class of microalgae, we may suggest that limpets in the exposed site have a diet richer in diatoms and dinoflagellates, whereas red algae predominate in the sheltered site. Green algae are probably an equally important component of the limpet's diet in both sites. In fact, a film of diatoms was typically observed in the exposed site during winter.

Interesting quantitative differences were found in the total amount of fatty acids, as well as in the main fatty acid classes present in male and female limpets in the exposed and sheltered sites. Females were seen to have a higher total FAME content than males in both sites. During summer, female limpets were predominantly composed of SFA and MUFA. This was probably caused by the more advanced stage of gonad development and possibly by better feeding conditions of the limpets during summer. In several tissues of the female bivalve dog cockle (*Glycymeris glycymeris*) SFA were also found to be the major class of fatty acids (Galap et al., 1999). Males, on the other hand, are richer in PUFA and HUFA (in relative terms, since the absolute amount is similar or even lower than in females). Therefore, male limpets resemble the *H. laevigata*, *H. rubra*, *O. edulis* and *C. gigas* profile (Abad et al., 1995; Dunstan et al., 1996; Pazos et al., 1996), with PUFA predominating over SFA, followed by MUFA.

It is not surprising that males have a low SFA and MUFA content and a higher PUFA level, given that male gametes are composed mostly of structural fatty acids. PUFA are typically found in high amounts in phospholipids, which are mainly structural components of membranes. SFA and MUFA, on the other hand, are usually an important fraction of neutral lipids, which normally function as an energy reserve. The male gonad has been found to be comparatively richer in polysaccharides and protein nitrogen (due to the high nucleic acid content of male gametes) than the female gonad (Blackmore, 1969a). Additionally, phospholipids have been shown to form a prominent component in spermatozoa of marine invertebrates (Lawrence and Giese, 1969; Cook and Gabbott, 1972). Female gametes, on the other hand, besides having phospholipids as part of their membranes, have also the role of providing energy for the developing embryo and are therefore richer in energetic reserves.

Blackmore (1969a) verified that the increase in the polysaccharide content of *P. vulgata* during maturation was significantly lower in the ovary than in the testis. On the other hand, while lipid accumulated in the gonad of both sexes during maturation, this accumulation was higher in the female gonad and body tissue, particularly in later

developmental stages. Therefore, the higher amount of total FAME found in female limpets in the present study confirmed previous observations in which the lipid content of the testes was found to be up to half of that in the ovaries (Simpson, 1982; Voogt, 1983; Wenne and Polak, 1989).

During winter a high variability in the amount of fatty acids was found among patches of the exposed site. This variability was probably related to the aggregation pattern of limpets at this time and did not enable the detection of significant differences between sites. Nevertheless, the results in Table 3 indicate the same general tendency for both sampling dates. In summer, limpets in the exposed site were characterised by having significantly higher absolute amounts of FAME, SFA, MUFA, PUFA, HUFA, ($n-3$) fatty acids and EPA than in the sheltered site. As for the fatty acid relative composition (% of total lipids, Fig. 2), exposed limpets were found to have similar or slightly higher SFA and MUFA and, consequently, a lower PUFA percentage than sheltered limpets. These results suggest that limpets in the exposed site have a higher amount of storage neutral lipids, which are naturally richer in SFA and MUFA. The limpets in the sheltered site, on the contrary, appear to be more depleted of lipid reserves, possibly having a higher phospholipid fraction, with a consequent increase in the PUFA relative amount. The triacylglycerol (neutral storage lipid) level in the scallop *P. maximus* has been shown to reflect not only the maturity state of the female gonad but also to give a reliable indication regarding local food conditions (Pazos et al., 1997).

4.3. Conclusions and hypotheses

In conclusion, the results obtained in this study show that the spatial aggregation strategy adopted by limpets in sites of great wave and wind exposure does not affect their feeding and reproductive success, at least in the site examined here. In fact, a greater gonad development, a higher GSI and an elevated total FAME content was found in the exposed site. Several possible explanations may be suggested as the cause for these observations. One possible explanation is that the limpets in the exposed site, besides having a qualitatively different diet than the sheltered population, have also access to a higher quantity of food and are, therefore, in a better dietary condition that allows them to invest more in reproduction, without deleterious effects on growth and survival. For instance, Underwood and Jernakoff (1984) noted that less algae grew in sheltered sites on the New South Wales coast, particularly during the warmer periods of the year (when there is less splash and spray effect from waves), while algal cover increased with increasing exposure to wave action. On the other hand, the feeding conditions have also been related to the gonad development in other species (Barnes et al., 1963; Simpson, 1982).

Secondly, some authors have attributed differences in the glycogen and polysaccharide levels to differences in the metabolic rate of the animals (Whyte, 1968 in Branch, 1981; Blackmore, 1969a). Therefore, it is conceivable that if the exposed limpets have a lower metabolic rate than the sheltered limpets, as a result of lower desiccation stress and reduced movement (feeding excursions), the energy saved may allow greater lipid storage.

Another possible explanation could be associated with the reproductive cycle of the populations. The annual reproductive cycle may well be related to the temperature cycle; Orton and Southward (1961) observed that the peak of *P. depressa* gonad development

was coincident with the highest temperatures. On the other hand, there are several theories concerning the spawning stimuli in *Patella* but the most credible is related to wave action or to onshore winds, associated with a direct mechanical shock (Orton et al., 1956; Orton and Southward, 1961). Since both sites were located in the same rocky shore, they were submitted to similar environmental conditions except for the magnitude of exposure. It is therefore conceivable that the population in the site submitted to a higher wave and wind exposure will be stimulated to spawn earlier. As a consequence, the whole population may attain the resting phase earlier in the season and start redeveloping their gonads sooner than the sheltered limpets. If this is true, the exposed population may be entrained to be more advanced in the reproductive cycle, in comparison with the sheltered population. If so, given that the samples were collected at the same time in both populations, the stage of gonad development and the reproductive status may not be comparable on the same temporal scale.

Finally, it should be mentioned that given the complexity of factors operating in the environment, the hypotheses discussed here may not be mutually excluding but a combination of several factors could be responsible for the results obtained in the present study.

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References

Abad, M., Ruiz, C., Martinez, D., Mosquera, G., Sánchez, J.L., 1995. Seasonal variations of lipid classes and fatty acids in flat oyster, *Ostrea edulis*, from San Cibran (Galicia, Spain). Comp. Biochem. Physiol. 110C, 109–118.

Barber, B.J., Blake, N.B., 1981. Energy storage and utilization in relation to gametogenesis in *Argopecten irridians concentricus* (Say). J. Exp. Mar. Biol. Ecol. 52, 121–134.

Barnes, H., Barnes, M., Finlayson, D.M., 1963. The seasonal changes in body weight, biochemical composition, and oxygen uptake in two common Boreo-Arctic cirripedes, *Balanus balanoides* and *B. balanus*. J. Mar. Biol. Assoc. U. K. 43, 185–211.

Bayne, B.L., Newell, R.C., 1983. Physiological energetics of marine molluscs. In: Saleuddin, A.S.M., Wilbur, K.M. (Eds.), The Mollusca, vol. 4. Academic Press, New York, pp. 407–515.

Blackmore, D.T., 1969a. Studies of *Patella vulgata* L.: II. Seasonal variation in biochemical composition. J. Exp. Mar. Biol. Ecol. 3, 231–245.

Blackmore, D.T., 1969b. Studies of *Patella vulgata* L.: I. Growth, reproduction and zonal distribution. J. Exp. Mar. Biol. Ecol. 3, 200–213.

Blight, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Boaventura, D., Cancela da Fonseca, L., Hawkins, S.J., 2002. Analysis of competitive interactions between the limpets *Patella depressa* Pennant and *Patella vulgata* L. in the northern coast of Portugal. J. Exp. Mar. Biol. Ecol. 271, 171–188.

Bonnet, J.C., Sidwell, V.D., Zook, E.G., 1974. Chemical and nutritive values of several fresh and canned finfish, crustaceans and mollusks: Part II. Fatty acid composition. Mar. Fish. Rev. 36, 8–14.

Branch, G.M., 1975a. Intraspecific competition in *Patella cochlear* Born. J. Anim. Ecol. 44, 263–282.
Branch, G.M., 1975b. Mechanisms reducing intraspecific competition in *Patella* spp.: migration, differentiation and territorial behaviour. J. Anim. Ecol. 44, 575–600.
Branch, G.M., 1981. The biology of limpets: physical factors, energy flow, and ecological interactions. Oceanogr. Mar. Biol. Ann. Rev. 19, 235–380.
Chuecas, L., Riley, J.P., 1969. Component fatty acids of the total lipids of some marine phytoplankton. J. Mar. Biol. Assoc. U. K. 49, 97–116.
Cook, P.A., Gabbott, P.A., 1972. Seasonal changes in the biochemical composition of the adult barnacle, *Balanus balanoides*, and the possible relationships between biochemical composition and cold-tolerance. J. Mar. Biol. Assoc. U. K. 52, 805–825.
Creese, R.G., Underwood, A.J., 1982. Analysis of inter- and intra-specific competition amongst intertidal limpets with different methods of feeding. Oecologia 53, 337–346.
Dunstan, G.A., Baillie, H.J., Barrett, S.M., Volkman, J.K., 1996. Effect of diet on the lipid composition of wild and cultured abalone. Aquaculture 140, 115–127.
Feare, C.J., 1970. Aspects of the ecology of an exposed shore population of dogwhelks *Nucella lapillus* (L.). Oecologia 5, 1–18.
Gabbott, P.A., 1983. Developmental and seasonal metabolic activities in marine molluscs. In: Hochachka, P.W. (Ed.), The Mollusca. Environmental Biochemistry and Physiology, vol. 2. Academic Press, New York, pp. 165–217.
Galap, C., Netchitaïlo, P., Leboulenger, F., Grillot, J.-P., 1999. Variations of fatty acid contents in selected tissues of the female dog cockle (*Glycymeris glycymeris* L., Mollusca, Bivalvia) during the annual cycle. Comp. Biochem. Physiol. 122A, 241–254.
Gardner, D., Riley, J.P., 1972. The component fatty acids of the lipids of some species of marine and freshwater molluscs. J. Mar. Biol. Assoc. U. K. 52, 827–838.
Guerra, M.T., Gaudêncio, M.J., 1986. Aspects of the ecology of *Patella* spp. on the Portuguese coast. Hydrobiologia 142, 57–69.
Lawrence, J.M., Giese, A.C., 1969. Changes in the lipid composition of the chiton, *Katharina tunicata*, with the reproductive and nutritional state. Physiol. Zool. 42, 353–360.
Metcalfe, L.D., Schmitz, A.A., 1961. The rapid preparation of fatty acid esters for gas chromatography analysis. Anal. Chem. 33, 363–364.
Orton, J.H., Southward, A.J., 1961. Studies on the biology of limpets: IV. The breeding of *Patella depressa* Pennant on the North Cornish coast. J. Mar. Biol. Assoc. U. K. 41, 653–662.
Orton, J.H., Southward, A.J., Dodd, J.M., 1956. Studies on the biology of limpets. II. The breeding of *Patella vulgata* L. in Britain. J. Mar. Biol. Assoc. U. K. 35, 149–176.
Pastoriza, L., Gallardo, J.M., Franco, J.M., Sampedro, G., 1981. Ácidos grasos de la almeja babosa *Venerupis pullastra* (Mont.), berberecho *Cerastoderma edule* (L.) y mejillón Mytilus galloprovincialis. Lam. Inv. Pesq. 45, 21–25.
Pazos, A.J., Ruíz, C., García-Martín, O., Abad, M., Sánchez, J.L., 1996. Seasonal variations of the lipid content and fatty acid composition of *Crassostrea gigas* cultured in El Grove, Galicia, N.W. Spain. Comp. Biochem. Physiol. 114B, 171–179.
Pazos, A.J., Román, G., Acosta, C.P., Sánchez, J.L., Abad, M., 1997. Lipid classes and fatty acid composition in the female gonad of *Pecten maximus* in relation to reproductive cycle and environmental variables. Comp. Biochem. Physiol. 117B, 393–402.
Ruiz, C., Abad, M., Sedano, F., Garcia-Martin, L.O., Sánchez López, J.L., 1992. Influence of seasonal environmental changes on the gamete production and biochemical composition of *Crassostrea gigas* (Thunberg) in suspended culture in El Grove, Galicia, Spain. J. Exp. Mar. Biol. Ecol. 155, 249–262.
Sargent, J., Henderson, R.J., Tocher, D.R., 1989. The lipids. In: Halver, J.E. (Ed.), Fish Nutrition, 2nd ed. Academic Press, London.
Shimma, Y., Taguchi, H., 1964. A comparative study on fatty acid composition of shellfish. Bull. Jap. Soc. Sci. Fish. 30, 153–159.
Simpson, R.D., 1982. Reproduction and lipids in the sub-Antarctic limpet *Nacella (Patinigera) macquariensis* Finlay, 1927. J. Exp. Mar. Biol. Ecol. 56, 33–48.
Stimson, J., 1970. Territorial behaviour of the owl limpet, *Lottia gigantea*. Ecology 51, 113–118.

Underwood, A.J., 1976. Food competition between age-classes in the intertidal neritacean *Nerita atramentosa* Reeve (Gastropoda: Prosobranchia). J. Exp. Mar. Biol. Ecol. 23, 145–154.

Underwood, A.J., 1984. Vertical and seasonal patterns in competition for microalgae between intertidal gastropods. Oecologia 64, 211–222.

Underwood, A.J., 1997. Experiments in Ecology. Their Logical Design and Interpretation Using Analysis of Variance. Cambridge Univ. Press, Cambridge. 504 pp.

Underwood, A.J., Jernakoff, P., 1984. The effects of tidal height, wave-exposure, seasonality and rock-pools on grazing and the distribution of intertidal macroalgae in New South Wales. J. Exp. Mar. Biol. Ecol. 75, 71–96.

Voogt, P.A., 1983. Lipids: their distribution and metabolism. In: Hochachka, P.W. (Ed.), The Mollusca. Metabolic Biochemistry and Molecular Biomechanics, vol. 1. Academic Press, New York, pp. 329–370.

Wenne, R., Polak, L., 1989. Lipid composition and storage in the tissues of the bivalve, *Macoma balthica*. Biochem. System. Ecol. 17, 583–587.

Whyte, J.N.C., Englar, J.R., Carswell, B.L., 1990. Biochemical composition and energy reserves in *Crassostrea gigas* exposed to different levels of nutrition. Aquaculture 90, 157–172.