ELSEVIER

Comparative Biochemistry and Physiology Part B 136 (2003) 425–441

CBP

www.elsevier.com/locate/cbpb

Spatial and temporal variation of the fatty acid composition of *Patella* spp. (Gastropoda: Prosobranchia) soft bodies and gonads

Sónia Brazão[a], Sofia Morais[a], Diana Boaventura[a,*], Pedro Ré[a], Luís Narciso[a], Stephen J. Hawkins[b,c]

[a]*Laboratório Marítimo da Guia/IMAR – Faculdade da Ciências de Universidade de Lisboa, Estrada do Guincho, Cascais 2750-374, Portugal*
[b]*Marine Biological Association of the UK, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, UK*
[c]*Division of Biodiversity and Ecology, School of Biological Sciences, Biomedical Sciences Building, University of Southampton, Southampton, SO16 7PX, UK*

Received 12 April 2003; received in revised form 22 July 2003; accepted 25 July 2003

Abstract

This study evaluated the effects of season and spatial distribution on the fatty acid composition of *Patella depressa* gonads and *Patella* spp. soft body tissue. The results show that 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) eicosapentaenoic acid (EPA 20:5(n-3)), and arachidonic acid (ARA 20:4(n-6)). *P. depressa* and *P. ulyssiponensis* soft body fatty acid profiles revealed significant differences between sexes; males showed significantly higher percentages of PUFA, highly unsaturated fatty acids (HUFA), (n-3) fatty acids and ARA, while in females significantly higher proportions of MUFA were found. Analysis of variance on the fatty acid composition of *P. depressa* gonads revealed significant differences between sexes, which were more marked than when the whole body was analysed. Males showed a significantly higher percentage of PUFA, HUFA, fatty acids from the (n-3) and (n-6) series, ARA and EPA, while females were seen to have higher proportions of SFA, MUFA and total fatty acid methyl esters (FAME). Some variability was seen to occur due to shore location and seasons, but these effects were not so obvious.

Keywords: Fatty acid composition; Gonads; Lipids; Molluscs; *Patella ulyssiponensis*; *Patella depressa*; *Patella rustica*; *Patella vulgata*; Soft body

1. Introduction

Lipids are major sources of metabolic energy and of essential materials for the formation of cell and tissue membranes (Sargent, 1995). They are very important in the physiology and reproductive processes of marine animals and reflect the special biochemical and ecological conditions of the marine environment (Pazos et al., 1997). Lipids also provide energy for growth during conditions of limited food supply, when carbohydrate levels (the main energetic reserve in molluscs) are low (Gabbott and Bayne, 1973; Ruiz et al., 1992; Abad et al., 1995; Pazos et al., 1996, 1997).

The lipid composition of molluscs can be affected by external (exogenous) factors, such as fluctuations in the environmental conditions and qualitative and/or quantitative changes in food availability, or by internal (endogenous) factors

---

*Corresponding author. Tel.: +351-21-4869211; fax: +351-21-4869720.
E-mail address: dianab@fc.ul.pt (D. Boaventura).

1096-4959/03/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S1096-4959(03)00243-4

such as sexual maturation (Gardner and Riley, 1972; Bonnet et al., 1974; Galap et al., 1999). Accumulation and depletion of stored reserves in molluscs depends mainly on the stage of gonad development, environmental factors affecting metabolic activities and on the quantity and nutritional value of the food supply (Gabbott, 1983; Whyte et al., 1990; Pazos et al., 1996). Glycogen is usually the major stored source of energy in molluscs (Barber and Blake, 1981), while lipids are considered as the nutritive reserve product of the gonads (Wenne and Polak, 1989). A strong correlation between the gonad lipid content and the phase of the reproductive cycle has been established in several bivalves and prosobranch species (Simpson, 1982; Abad et al., 1995; Pazos et al., 1996, 1997).

Seasonal variations in lipid and fatty acid composition have been reported for several marine molluscs (Taylor and Venn, 1979; Gabbott, 1983; Beninger and Stephan, 1985; Kluytmans et al., 1985; Piretti et al., 1987, 1988; Chu et al., 1988, 1990; Hayashi and Kishimura, 1991; Ruiz et al., 1992; Abad et al., 1995; Pazos et al., 1996, 1997) and are generally related to the growth-maturation cycle: in the summer when the growth phase takes place, reserves of lipids are build up and stored, and these are later mobilised for gametogenesis in the maturation phase (often autumn or winter), being normally lost during spawning. However, the majority of these publications have focussed on bivalves, probably as a result of their great commercial importance and impact on public health. Studies on the biochemical composition, particularly in terms of fatty acid profiles, of prosobranch gastropods are scarce (but see Voogt, 1983; Dunstan et al., 1996).

Limpets are herbivorous grazers that remove large quantities of unicellular microbes, algal germlings and detritus, apparently unselectively, during feeding excursions around the home scar. As a consequence, there is a considerable variation in their diets (Fretter and Graham, 1962, 1976; Branch, 1981). There is a large amount of literature detailing the fatty acid composition of many species of marine algae (Chuecas and Riley, 1969; Ben-Amotz et al., 1987; Sargent et al., 1989; Sukenik et al., 1993; Brown et al., 1997). Availability and quality of algal lipids are very important in the nutrition, growth and development of aquatic animals such as marine fish larvae, shrimps and molluscs (Sukenik et al., 1993). Certain fatty acids present in algae are considered nutritionally essential for most marine species, affecting their growth and condition (Pazos et al., 1997).

Seasonal changes in biochemical composition of the limpet *P. vulgata* Linnaeus, 1758 have been investigated by Blackmore (1969). In this species, the gonad develops during late summer, with spawning occurring in autumn and early winter. The level of lipids reaches a minimum in winter, and gradually increases during the spring and early summer when gonad maturation takes place. During this period lipids accumulate in the gonad, while the level in the gonad-free tissue begins to fall (Blackmore, 1969). No similar data has been collected so far for *P. depressa* Pennant, 1777. One of the purposes of this study is to provide for the first time the description of the fatty acid composition of the gonads of this species, at two different stages of development, as well as of the temporal changes (winter vs. summer) occurring in this organ. In addition, this work presents a complete description of the fatty acid profile of *P. ulyssiponensis* Gmelin, 1791; *P. vulgata* and *P. rustica* Linnaeus, 1758. Finally, we used an experimental design to investigate the effects of ecological factors on the fatty acid composition of male and female limpets.

Thus, the present study aims to: (1) describe qualitatively the body fatty acid composition of four different species of *Patella* spp. (*P. depressa*, *P. ulyssiponensis*, *P. vulgata* and *P. rustica*); (2) compare the soft body fatty acid composition of *P. depressa* and *P. aspera* at different shore levels and (3) analyse differences between the biochemical composition of *P. depressa* gonads, during summer and winter periods, in different stages of gonad development.

2. Material and methods

2.1. Sample collection and preparation

2.1.1. Fatty acid composition of Patella spp. soft body

Samples were collected from two rocky shores of the central region of the Portuguese coast, Avencas (*P. depressa*, *P. ulyssiponensis* and *P. vulgata*) and Cabo Raso (*P. rustica*, which is not found in Avencas). Two areas were sampled within each zone of the vertical range of each species: *P. depressa* was collected from the upper and lower mid-shore zone and *P. ulyssiponensis* from the low shore and lower mid-shore zone. The lower mid-shore corresponded to the zone of overlap where both *P. depressa* and *P. ulyssiponensis* were present (hereafter designated by 'overlapping zone'). The upper mid-shore, where only *P. depressa* occurs, and the low shore dominated by *P. ulyssiponensis* corresponded to the zones where there was no overlap between species (and designated by 'non overlapping zone').

Only one area was established within each zone for *P. vulgata* (upper and lower mid-shore zone), since this species was not found in sufficient numbers. *P. rustica* was collected from 2 areas in the mid-shore zone from Cabo Raso. At least 100 individuals per species were collected, during spring low tide, within each area. After preliminary studies, October was the month chosen for sample collection, because it was one of the months of highest gonad maturation in *P. depressa*.

Triplicates of males and females, of similar lengths and in stage IV of gonad development (due to the insufficient number of individuals found in the most advanced stage V), were collected for each species and shore-zone, and used for fatty acid analysis. Thus, biochemical analyses were conducted on 24 individuals (12 males and 12 females) of *P. depressa* and *P. ulyssiponensis*, providing soft-body profiles that could be compared statistically. Twelve individuals of *P. vulgata* and *P. rustica* (six males and six females) were analysed and compared qualitatively, due to the small sample size.

2.1.2. Fatty acid composition of *P. depressa* gonads

To analyse the temporal variation in the fatty acid composition of *P. depressa* gonads, eight areas of 4 m^2 each were marked at the Avencas upper mid-shore. Four areas were sampled in summer (September) and the remaining four in winter (January and February), on two random sampling dates in each period. One hundred limpets above 20 mm (with differentiated gonads) were collected in each area and immediately frozen at −20 °C. Gonads were later removed from the shells and grouped according to their maturation stage. Given the small sample size of the gonads, several individuals of the same sex and gonad stage of development had to be pooled and triplicate samples were used for fatty acid analysis. This kind of procedure does not enable a distinction between developing and spawning stages since the samples had to be maintained frozen for fatty acid analysis. In this study, approximately 24 individuals (males and females) were analysed per stage of gonad development (stages II and III).

2.2. Laboratorial procedure and fatty acid analysis

In both sets of observations, the shell length of each animal was measured with a vernier calliper to the nearest millimetre, and the sex and stage of gonad development was assessed by cutting away the foot from most of its attachment to the visceral mass and shell, and turning it forward (Orton et al., 1956; Orton and Southward, 1961). Colour was used to assess the sex, the male gonad being pinkish white or cream and the female brownish or dark green. The stage of gonad development was macroscopically identified according to the stages defined by Orton et al. (1956) for *P. vulgata*.

Soft bodies and gonads (semi-frozen) were removed from the shell and stored in pre-weighted 'Eppendorf'-type tubes and then stored in liquid nitrogen for later analysis of fatty acids. After freeze-drying in a Savant VP100® (24 h), samples were ground in a Potter homogenizer with chloroform–methanol–water (2:2:1.8), according to the procedure of Bligh 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. Gas–liquid chromatography (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 reference well-characterised cod liver oil chromatograms (Christie, 1982; Narciso, 1996).

2.3. Data analysis and experimental design

Each experimental treatment was analysed in triplicate. Data were subjected to analysis of variance using a 4-way mixed model ANOVA. In order to eliminate quantitative differences between

Table 1
Fatty acid composition (% of total FAME) of *P. depressa* and *P. ulyssiponensis* soft body ($n=6$ individuals per sex per zone); in bold are the sum of the classes

Fatty acids % total FAME	*P. ulyssiponensis* Low-shore		Lower mid-shore		*P. depressa* Lower mid-shore		Upper mid-shore	
	Male	Female	Male	Female	Male	Female	Male	Female
13:0	0.04±0.01	0.04±0.01	0.10±0.01	0.06±0.00	0.13±0.01	0.06±0.02	0.11±0.02	0.17±0.05
14:0	5.25±0.45	6.17±0.16	5.44±0.26	7.11±0.28	5.50±0.51	7.33±0.48	5.25±0.47	6.26±0.23
15:0	1.48±0.07	1.14±0.07	1.62±0.19	1.63±0.14	1.15±0.05	0.89±0.03	0.90±0.08	0.81±0.06
16:0	19.35±0.61	20.39±0.88	23.12±0.79	25.72±0.31	17.18±1.02	24.49±1.93	16.30±1.54	18.92±0.80
17:0	1.00±0.05	0.74±0.04	1.06±0.20	1.10±0.11	0.86±0.03	0.62±0.07	0.74±0.05	0.64±0.05
18:0	5.08±0.31	4.87±0.33	5.04±0.70	5.11±0.13	5.63±0.21	4.61±0.24	5.75±0.30	5.87±0.30
20:0	1.13±0.37	0.21±0.01	0.68±0.14	0.36±0.09	0.51±0.07	0.31±0.04	0.39±0.11	0.38±0.09
22:0	0.04±0.00	0.10±0.02	0.03±0.03	0.02±0.01	0.00±0.00	0.08±0.08	0.31±0.28	0.18±0.10
Σ SFA	**33.35±0.83**	**33.64±1.01**	**37.07±1.68**	**41.11±0.51**	**30.95±1.45**	**38.17±2.28**	**29.74±1.96**	**32.26±1.05**
14:1(n-5)	0.00±0.00	0.05±0.02	0.02±0.02	0.06±0.03	0.00±0.00	0.13±0.04	0.03±0.03	0.07±0.01
16:1(n-7)	2.02±0.48	2.40±0.34	1.68±0.54	1.84±0.05	2.26±0.40	3.32±0.28	3.01±0.33	3.57±0.23
16:1(n-5)	0.62±0.18	0.55±0.15	0.52±0.12	0.49±0.07	1.04±0.11	0.83±0.08	1.39±0.13	1.46±0.14
17:1(n-8)	0.07±0.02	0.04±0.01	0.09±0.02	0.03±0.02	0.15±0.02	0.01±0.01	0.23±0.03	0.20±0.07
18:1(n-9)	5.04±0.55	5.91±0.38	6.87±1.12	6.91±0.32	5.27±0.30	8.29±0.59	5.04±0.21	6.01±0.47
18:1(n-7)	7.74±0.22	8.96±0.15	8.18±0.27	9.48±0.34	10.71±0.37	11.17±0.34	11.00±0.78	12.28±0.25
18:1(n-5)	0.03±0.00	0.04±.0.00	0.04±0.01	0.00±0.00	0.01±0.01	0.00±0.00	0.04±0.02	0.11±0.08
20:1(n-9)	2.49±0.52	2.57±0.30	1.99±0.26	2.38±0.30	2.91±0.30	4.41±0.63	1.74±0.05	3.96±0.28
20:1(n-7)	1.51±0.09	1.80±0.03	2.03±0.46	1.78±0.12	1.72±0.13	2.05±0.12	2.80±0.34	2.16±0.11
20:1(n-5)	0.43±0.04	0.48±0.02	0.61±0.14	0.47±0.05	0.90±0.18	0.53±0.04	1.18±0.09	0.69±0.05
22:1(n-11)	0.07±0.03	0.14±0.01	0.11±0.04	0.05±0.02	0.16±0.06	0.49±0.10	0.46±0.45	0.46±0.32
22:1(n-9)	2.08±0.37	2.13±0.08	1.85±0.58	3.08±0.46	1.66±0.37	2.62±0.13	2.58±0.35	2.15±0.57
24:1(n-9)	0.02±0.02	0.14±0.04	0.06±0.02	0.34±0.10	0.00±0.00	0.00±0.00	0.10±0.10	0.11±0.07
24:1(n-7)	0.03±0.03	0.19±0.08	0.04±0.02	0.26±0.06	0.00±0.00	0.00±0.00	0.16±0.15	0.10±0.05
Σ MUFA	**22.12±0.55**	**25.37±0.57**	**24.07±1.99**	**27.13±1.53**	**26.78±0.88**	**33.83±1.63**	**29.73±1.35**	**33.28±1.10**
Iso 15:0	0.18±0.07	0.07±0.01	0.24±0.05	0.13±0.04	0.26±0.05	0.11±0.03	0.23±0.02	0.18±0.03
Anteiso 15:0	0.03±0.01	0.05±0.00	0.04±0.04	0.00±0.00	0.05±0.03	0.03±0.02	0.07±0.00	0.08±0.05
Iso 16:0	0.20±0.02	0.16±0.02	0.22±0.04	0.22±0.03	0.64±0.06	0.30±0.08	0.87±0.06	0.89±0.12
Anteiso 16:0	0.19±0.06	0.10±0.00	0.23±0.14	0.03±0.00	0.24±0.04	0.04±0.01	0.14±0.02	0.10±0.03
Iso 17:0	0.26±0.06	0.15±0.04	0.24±0.08	0.16±0.00	0.38±0.05	0.30±0.05	0.54±0.05	0.56±0.08
Anteiso 17:0	0.19±0.05	0.20±0.05	0.18±0.06	0.15±0.02	0.67±0.09	0.12±0.09	0.87±0.11	0.91±0.21
Iso 18:0	0.39±0.18	0.06±0.05	0.65±0.32	0.85±0.28	2.34±0.61	2.02±0.60	1.75±0.11	1.55±0.76
Σ Branched	**1.41±0.31**	**0.77±0.13**	**1.76±0.33**	**1.54±0.34**	**4.58±0.62**	**2.89±0.80**	**4.47±0.25**	**4.27±0.60**
16:3(n-4)	0.46±0.14	0.57±0.07	0.50±0.09	0.15±0.09	0.27±0.15	0.10±0.05	0.57±0.25	0.31±0.21
16:3(n-3)	0.00±0.00	0.00±0.00	0.01±0.01	0.00±0.00	0.05±0.05	0.00±0.00	0.00±0.00	0.28±0.16
16:4(n-3)	0.28±0.07	0.35±0.07	0.30±0.09	0.17±0.04	0.38±0.02	0.19±0.01	0.52±0.03	0.41±0.13
16:4(n-1)	0.93±0.30	0.58±0.04	0.77±0.40	0.48±0.06	0.47±0.12	0.24±0.05	0.27±0.05	0.34±0.15
18:2(n-6)	0.77±0.20	0.99±0.15	1.17±0.31	0.48±0.09	1.38±0.16	0.59±0.11	2.08±0.22	2.30±0.59
18:3(n-3)	2.66±0.35	2.70±0.38	3.08±0.55	1.85±0.20	3.62±0.13	1.68±0.27	2.89±0.27	3.01±1.00
18:4(n-3)	1.12±0.20	1.28±0.23	0.88±0.29	0.54±0.08	0.43±0.09	0.27±0.06	0.23±0.07	0.19±0.05
20:2(n-6)	1.83±0.16	2.12±0.10	1.97±0.21	1.77±0.20	1.68±0.12	1.57±0.15	1.60±0.17	1.77±0.14
20:3(n-6)	0.66±0.04	0.84±0.04	0.59±0.09	0.66±0.04	0.49±0.05	0.39±0.06	1.07±0.16	0.99±0.19
20:4(n-6)	8.56±0.73	5.73±0.27	10.31±1.07	5.75±0.49	14.21±1.17	8.52±2.13	11.33±0.99	9.50±1.62
20:3(n-3)	2.67±0.14	2.75±0.24	1.95±0.47	1.57±0.24	2.82±0.14	2.22±0.24	2.36±0.26	2.08±0.37
20:4(n-3)	1.22±0.15	1.93±0.13	0.88±0.19	0.80±0.17	0.52±0.05	0.59±0.16	1.13±0.27	0.82±0.27
20:5(n-3)	19.74±1.09	17.04±0.66	13.60±1.27	12.28±1.30	10.45±0.51	7.31±0.72	9.08±1.21	6.35±0.95
21:5(n-3)	0.54±0.15	0.52±0.06	0.06±0.03	0.56±0.15	0.05±0.01	0.14±0.09	0.79±0.12	0.43±0.15
22:4(n-6)	0.35±0.11	0.59±0.04	0.12±0.01	0.71±0.15	0.37±0.06	0.81±0.24	0.11±0.11	0.23±0.06
22:5(n-6)	0.13±0.05	0.23±0.01	0.08±0.03	0.30±0.07	0.03±0.02	0.17±0.06	0.79±0.28	0.57±0.24
22:5(n-3)	0.98±0.11	1.59±0.12	0.65±0.25	1.35±0.54	0.37±0.03	0.26±0.12	0.70±0.35	0.30±0.13
22:6(n-3)	0.27±0.03	0.46±0.04	0.24±0.12	0.89±0.20	0.16±0.02	0.11±0.01	0.53±0.37	0.37±0.11
Σ PUFA	**43.13±0.73**	**40.23±0.90**	**37.09±2.81**	**30.22±1.21**	**37.71±1.28**	**25.12±3.13**	**36.07±3.37**	**30.21±1.83**
Σ HUFA	**36.93±0.88**	**33.78±0.93**	**30.41±1.69**	**26.56±1.04**	**31.12±1.42**	**22.07±2.83**	**29.49±3.20**	**23.38±1.22**

Table 1 *(Continued)*

Fatty acids % total FAME	*P. ulyssiponensis*				*P. depressa*			
	Low-shore		Lower mid-shore		Lower mid-shore		Upper mid-shore	
	Male	Female	Male	Female	Male	Female	Male	Female
Σ(n-3)	**29.46±1.47**	**28.60±1.02**	**21.61±2.51**	**20.00±1.27**	**18.84±0.62**	**12.74±1.49**	**18.19±2.39**	**14.23±2.38**
Σ(n-6)	**12.28±0.81**	**10.48±0.32**	**14.19±1.20**	**9.60±0.10**	**18.15±1.28**	**12.04±2.48**	**16.96±1.23**	**15.34±1.18**
Σ(n-3)/Σ(n-6)	**2.46±0.28**	**2.77±0.16**	**1.59±0.21**	**2.14±0.13**	**1.09±0.10**	**1.14±0.21**	**1.07±0.10**	**0.96±0.24**
DHA/EPA	0.02±0.00	0.03±0.00	0.02±0.01	0.09±0.02	0.02±0.00	0.02±0.00	0.05±0.04	0.05±0.01
Σ Total FAME (μg/mg DW)	**41.55±11.44**	**67.96±2.58**	**47.59±16.35**	**28.95±5.47**	**24.69±2.94**	**39.07±7.50**	**26.30±3.29**	**48.58±13.68**

Data are means±S.E.

the samples, the fatty acid composition was expressed in relative terms, as a percentage of the total fatty acid methyl esters (FAME). The data submitted to analysis of variance were: total FAME, the main classes of fatty acids—saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), total fatty acids from the (n-3) and (n-6) series—as well as the most abundant PUFAs, eicosapentaenoic acid (EPA, 20:5(n-3)) and arachidonic acid (ARA, 20:4(n-6)).

2.3.1. Fatty acid composition of Patella spp. soft body

This analysis compared the fatty acid composition of *P. depressa* and *P. ulyssiponensis* soft bodies from different shore levels.

The factors tested were ‘species’ (fixed, orthogonal, 2 levels—*P. depressa* and *P. ulyssiponensis*), ‘sex’ (fixed, orthogonal, two levels—male and female), ‘zone’ (fixed, orthogonal, 2 levels—overlapping and non-overlapping zone) and ‘area’ (random, nested within zone, 2 levels).

2.3.2. Fatty acid composition of P. depressa gonads

Temporal variation in the fatty acid composition of *P. depressa* gonads was studied for two different stages of gonad development (stages II and III). The factors tested were ‘sex’ (fixed, orthogonal, 2 levels), ‘season’ (fixed, orthogonal, two levels), ‘date’ (random, nested in season, 2 levels) and ‘area’ (random, nested within season and date, 2 levels).

Cochran’s *C*-test was used to check homogeneity of variance. When assumption of homogeneity of variance was violated, arcsine and logarithmic transformations were used (Underwood, 1997). 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. Fatty acid composition of Patella spp. soft body

The analysis of the fatty acid composition of the *Patella* spp. soft body revealed no important qualitative differences in the fatty acid profiles of the four limpet species (*P. depressa*, *P. ulyssiponensis*, *P. vulgata* and *P. rustica*, Tables 1 and 2). Quantitatively, the most important fatty acids of the total lipids of all the studied species were the SFA 14:0, 16:0 and 18:0; the MUFA 16:1(n-7), 18:1(n-7), 18:1(n-9) and 20:1(n-9), and the PUFA, ARA and EPA (see Tables 1 and 2). Palmitic acid (16:0) was the most abundant SFA found in all four species, with myristic (14:0) and stearic (18:0) acids being present in lower amounts.

The results of the statistical analysis conducted on *P. ulyssiponensis* and *P. depressa* (Table 3) revealed significant differences between male and female soft bodies for MUFA, PUFA, HUFA, total (n-3) fatty acids and ARA from all areas. This was also found for FAME and EPA in some zones and areas (Table 3, SNK tests). Significantly higher percentages of MUFA and total FAME were found in females. PUFA, HUFA, (n-3) fatty acids, ARA and EPA were significantly higher in males. No significant differences were found for SFA and (n-6) fatty acids.

The comparison of the *P. depressa* and *P. ulyssiponensis* soft-body fatty acid profiles revealed significant differences between these species, concerning their MUFA, HUFA, (n-3) and (n-6) fatty acids, ARA and EPA content. Nonetheless, it should be noted that in all of these classes, with the exception of (n-3) fatty acids, the differences

Table 2
Fatty acid composition (% of total FAME) of *P. vulgata* and *P. rustica* soft body ($n=3$ individuals per sex per zone); in bold are the sum of the classes

Fatty acids % total FAME	*P. vulgata*				*P. rustica*			
	Lower mid-shore		Upper mid-shore		Mid-shore area 1		Mid-shore area 2	
	Male	Female	Male	Female	Male	Female	Male	Female
13:0	0.08±0.02	0.10±0.05	0.06±0.01	0.10±0.03	0.37±0.02	0.43±0.07	0.33±0.04	0.13±0.06
14:0	5.89±0.63	4.66±0.87	5.49±0.16	5.60±0.29	5.71±0.61	6.35±1.18	6.00±1.04	6.54±0.66
15:0	1.24±0.02	0.81±0.01	1.29±0.19	1.43±0.27	1.23±0.25	1.04±0.07	1.22±0.11	1.45±0.30
16:0	18.40±2.29	14.51±2.02	18.52±1.25	17.45±0.76	16.90±3.03	19.96±4.46	17.69±2.73	18.63±2.77
17:0	0.73±0.05	0.75±0.13	0.88±0.06	0.80±0.15	0.70±0.17	0.53±0.06	0.75±0.11	0.88±0.18
18:0	4.03±0.32	5.04±0.55	5.83±0.48	4.53±0.20	5.20±0.51	4.31±0.23	5.08±0.63	4.12±0.22
20:0	0.29±0.06	0.46±0.10	0.27±0.02	0.24±0.02	0.44±0.15	0.45±0.19	0.41±0.09	0.26±0.02
22:0	0.00±0.00	0.01±0.01	0.04±0.02	0.05±0.03	0.03±0.03	0.07±0.03	0.00±0.00	0.02±0.01
Σ SFA	**30.67±3.18**	**26.35±2.10**	**32.35±1.48**	**30.19±0.82**	**30.59±3.16**	**33.14±5.59**	**31.48±2.90**	**32.02±2.79**
14:1(n-5)	0.09±0.05	0.03±0.03	0.05±0.03	0.04±0.02	0.04±0.04	0.07±0.03	0.03±0.03	0.01±0.01
16:1(n-7)	2.85±0.13	2.49±0.26	2.43±0.38	2.93±0.18	2.53±0.21	2.38±0.44	2.33±0.31	2.63±0.51
16:1(n-5)	0.55±0.07	0.94±0.11	0.52±0.08	0.66±0.28	0.94±0.27	0.58±0.04	0.80±0.13	0.89±0.05
17:1(n-8)	0.48±0.20	0.15±0.00	0.00±0.00	0.08±0.03	0.27±0.05	0.26±0.08	0.36±0.11	0.08±0.02
18:1(n-9)	11.60±0.75	6.97±3.15	10.46±0.89	8.97±0.93	4.39±1.28	5.58±1.33	4.22±1.32	7.01±0.63
18:1(n-7)	8.12±1.25	8.54±0.68	9.39±0.35	9.55±1.33	13.66±1.29	12.11±1.01	12.75±0.57	10.08±0.91
18:1(n-5)	0.02±0.02	0.05±0.05	0.03±0.02	0.04±0.02	0.17±0.07	0.02±0.02	0.69±0.44	1.56±0.42
20:1(n-9)	2.21±0.29	3.28±0.65	3.38±0.57	2.80±1.11	5.23±0.68	5.22±0.59	4.88±0.42	3.73±0.95
20:1(n-7)	1.95±0.35	1.75±0.16	2.59±0.14	1.96±0.29	1.70±0.29	1.96±0.20	1.57±0.37	1.84±0.16
20:1(n-5)	0.47±0.03	0.61±0.12	0.54±0.04	0.59±0.10	1.11±0.17	1.04±0.14	0.68±0.19	0.61±0.12
22:1(n-11)	0.18±0.09	0.07±0.07	0.11±0.05	0.10±0.04	0.10±0.10	0.45±0.17	0.01±0.01	0.14±0.04
22:1(n-9)	2.17±0.71	2.50±0.31	2.85±0.25	1.73±0.51	1.99±0.81	1.79±0.63	2.67±0.37	1.91±0.44
24:1(n-9)	0.09±0.02	0.24±0.12	0.18±0.09	0.05±0.02	0.05±0.03	0.18±0.03	0.04±0.04	0.12±0.07
24:1(n-7)	0.21±0.08	0.72±0.52	0.38±0.16	0.14±0.06	0.03±0.03	0.18±0.03	0.05±0.05	0.07±0.04
Σ MUFA	**31.00±3.73**	**28.35±2.81**	**32.92±0.50**	**29.64±2.47**	**32.23±2.16**	**31.81±2.65**	**30.98±2.02**	**30.68±1.40**
Iso 15:0	0.09±0.02	0.19±0.06	0.08±0.02	0.14±0.06	0.44±0.21	0.17±0.07	0.33±0.10	0.25±0.05
Anteiso 15:0	0.05±0.02	0.10±0.05	0.03±0.01	0.07±0.04	0.03±0.03	0.09±0.05	0.11±0.05	0.14±0.01
Iso 16:0	0.27±0.05	0.55±0.27	0.16±0.05	0.31±0.09	1.26±0.13	1.34±0.05	1.44±0.10	0.66±0.22
Anteiso 16:0	0.12±0.06	0.26±0.06	0.10±0.04	0.19±0.04	0.37±0.12	0.28±0.07	0.53±0.18	0.17±0.04
Iso 17:0	0.21±0.01	0.41±0.05	0.16±0.04	0.26±0.09	0.56±0.08	0.45±0.02	0.52±0.03	0.38±0.06
Anteiso 17:0	0.28±0.08	0.72±0.07	0.16±0.02	0.31±0.09	1.17±0.05	1.18±0.25	1.24±0.25	0.43±0.01
Iso 18:0	0.07±0.02	2.32±0.78	0.01±0.01	0.21±0.08	1.72±0.56	1.09±0.68	2.85±0.96	0.32±0.08
Σ Branched	**1.09±0.21**	**4.55±1.19**	**0.71±0.16**	**1.49±0.27**	**5.54±0.65**	**4.62±0.93**	**7.01±1.50**	**2.36±0.12**
16:3(n-4)	0.39±0.21	0.00±0.00	0.74±0.10	0.60±0.06	0.00±0.00	0.14±0.07	0.00±0.00	0.70±0.11
16:3(n-3)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.05±0.04	0.07±0.03	0.00±0.00	0.09±0.04
16:4(n-3)	0.20±0.03	0.29±0.03	0.25±0.02	0.30±0.10	0.52±0.10	0.46±0.06	0.60±0.08	0.37±0.06
16:4(n-1)	0.32±0.14	0.22±0.05	0.32±0.03	0.39±0.07	0.47±0.25	0.38±0.10	0.24±0.06	0.32±0.05
18:2(n-6)	2.17±0.27	2.05±0.62	2.28±0.34	1.69±0.18	2.27±0.17	2.45±0.24	1.49±0.65	0.05±0.01
18:3(n-3)	2.15±0.31	2.86±0.31	2.01±0.02	2.49±0.42	1.40±0.22	1.41±0.37	1.07±0.09	2.58±0.48
18:4(n-3)	0.64±0.18	0.45±0.22	0.53±0.03	0.64±0.28	0.19±0.11	0.23±0.03	0.17±0.09	0.54±0.07
20:2(n-6)	3.28±0.20	2.55±0.65	3.48±0.61	2.08±0.07	1.38±0.13	1.95±0.24	1.63±0.16	2.15±0.27
20:3(n-6)	0.92±0.11	1.02±0.07	1.07±0.15	0.96±0.12	1.11±0.18	1.80±0.20	0.80±0.33	1.05±0.33
20:4(n-6)	8.42±1.37	13.48±3.67	5.20±0.53	8.08±2.31	9.75±1.94	8.43±0.68	9.20±0.56	10.10±1.42
20:3(n-3)	1.98±0.20	2.05±0.53	2.24±0.04	2.05±0.70	0.58±0.29	1.16±0.68	1.23±0.03	1.80±0.43
20:4(n-3)	0.95±0.13	0.83±0.35	1.23±0.22	1.02±0.42	0.29±0.09	0.76±0.28	0.72±0.10	0.60±0.15
20:5(n-3)	13.19±4.71	10.18±2.21	11.46±1.12	15.92±3.36	11.65±3.59	7.48±4.12	11.17±3.46	12.58±3.37
21:5(n-3)	0.48±0.17	0.71±0.23	0.31±0.15	0.31±0.16	0.65±0.33	1.17±0.55	0.48±0.26	0.45±0.39
22:4(n-6)	0.40±0.14	0.42±0.19	0.49±0.06	0.31±0.03	0.18±0.18	0.37±0.25	0.69±0.54	0.22±0.05
22:5(n-6)	0.27±0.14	0.81±0.42	0.23±0.02	0.24±0.18	0.67±0.41	0.49±0.28	0.54±0.30	0.35±0.21
22:5(n-3)	1.10±0.36	1.26±0.50	1.61±0.03	1.17±0.18	0.25±0.19	0.92±0.41	0.32±0.19	0.90±0.04
22:6(n-3)	0.35±0.14	1.03±0.46	0.56±0.10	0.44±0.20	0.23±0.15	0.77±0.37	0.18±0.09	0.40±0.13
Σ PUFA	**37.25±6.71**	**40.75±3.77**	**34.02±0.83**	**38.67±3.33**	**31.64±4.39**	**30.43±7.13**	**30.53±3.42**	**34.94±3.94**
Σ HUFA	**31.37±5.85**	**34.88±4.80**	**27.89±0.81**	**32.56±3.56**	**26.73±4.41**	**25.29±6.75**	**26.96±2.93**	**30.59±3.56**

Table 2 *(Continued)*

Fatty acids % total FAME	*P. vulgata*				*P. rustica*			
	Lower mid-shore		Upper mid-shore		Mid-shore area 1		Mid-shore area 2	
	Male	Female	Male	Female	Male	Female	Male	Female
Σ(n-3)	**21.06±5.01**	**20.19±1.40**	**20.21±0.92**	**24.33±3.20**	**15.81±3.49**	**14.42±6.67**	**15.94±2.94**	**20.00±2.96**
Σ(n-6)	**15.47±1.50**	**20.34±2.65**	**12.75±0.47**	**13.36±1.98**	**15.36±2.15**	**15.49±0.77**	**14.35±0.61**	**13.91±1.33**
Σ(n-3)/Σ(n-6)	**1.33±0.19**	**1.01±0.09**	**1.59±0.12**	**1.91±0.40**	**1.07±0.29**	**0.90±0.38**	**1.10±0.18**	**1.43±0.16**
DHA/EPA	0.04±0.02	0.09±0.04	0.05±0.01	0.04±0.02	0.04±0.04	0.11±0.02	0.03±0.02	0.05±0.03
Σ Total FAME (μg/mg DW)	**39.12±8.30**	**27.14±20.85**	**62.76±6.50**	**43.38±1.02**	**28.13±7.05**	**19.61±2.45**	**26.95±7.85**	**45.12±9.34**

Data are means±S.E.

in fatty acid composition were dependent on the area (Table 3, SNK tests) and for EPA an interaction between species, sex and area was found. No significant differences between species were observed for SFA, PUFA and FAME. *P. ulyssiponensis* showed significantly higher percentages of HUFA, (n-3) fatty acids and EPA, while in *P. depressa* significantly higher contents of MUFA, (n-6) fatty acids and ARA were present, although not in all of the sampled areas (Table 3).

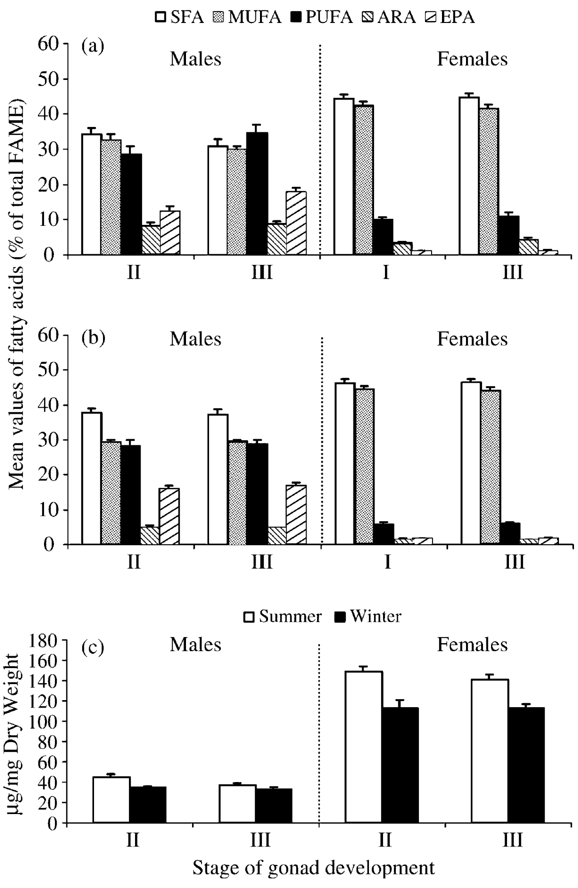

Fig. 1. Fatty acid composition (% of total FAME) of the male and female *P. depressa* gonad, in the different stages of development during summer (a) and winter (b). Total FAME concentrations (c) are expressed in micrometer/milligram DW (II = stage of development II and III = stage of development III).

Significant differences between zones were only found for PUFA and (n-3) fatty acids, with limpets in the non-overlapping zones (upper mid-shore + low shore) having significantly higher amounts of PUFA and (n-3) fatty acids than in the overlapping zone (lower mid-shore).

Another interesting result, common to all the analysed species, was the presence of very low amounts of 22:6(n-3) (docosahexaenoic acid-DHA) in both sexes.

3.2. Fatty acid composition of *P. depressa* gonads

The fatty acid composition of *P. depressa* gonads during the summer and winter periods is shown in Table 4. The analysis revealed that the fatty acids 14:0, 16:0, 18:0, 16:1(n-7), 18:1(n-9), 18:1(n-7), 20:1(n-9), 20:1(n-7), ARA and EPA were, in general, the major components of the gonads in both seasons. Again, DHA was found in very low amounts.

Different female and male fatty acid profiles were found for *P. depressa* gonads. Female gonads from the summer showed higher percentages of ARA than EPA, while in the male the opposite was found. The total FAME content was always 3–4 times greater in females than in males, this trend being observed in both seasons and in all stages of gonad development (Fig. 1).

The results of the statistical analysis conducted for stage II (Table 5) and III (Table 6) gonads revealed significant differences between male and female gonads. Female gonads presented signifi-

Table 3
Analysis of variance on the fatty acid composition (% of total FAME) of *P. ulyssiponensis* and *P. depressa* soft body

Source of variation	d.f.	SFA		MUFA		PUFA		HUFA		FAME		(n-3)		(*n*−6)		ARA		EPA	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Species = Sp	1	148.40	8.87 ns	466.44	12.47 ns	349.00	7.31 ns	351.54	4.08 ns	1686.02	1.96 ns	954.35	30.88 *	189.57	5.14 ns	130.91	2.48 ns	651.66	43.07 *
Sex = Se	1	148.47	8.75 ns	214.84	39.33 *	597.21	27.59 *	366.86	92.01 *	1480.30	224.58 **	117.66	69.67 *	149.85	18.06 ns	166.92	109.29 **	73.16	109.40 **
Zone = Zo	1	251.99	15.31 ns	1.30	0.42 ns	285.43	68.47 *	134.00	5.71 ns	1458.61	2.15 ns	224.68	104.36 **	0.83	0.50 ns	10.09	1.68 ns	55.21	2.74 ns
Area = Ar (Zo)	2	16.45	2.29 ns	3.12	0.55 ns	4.17	0.27 ns	23.46	2.16 ns	677.60	2.31 ns	2.15	0.21 ns	1.69	0.30 ns	6.02	1.21 ns	20.15	5.47 **
Sp × Se	1	21.95	4.77 ns	13.81	0.93 ns	56.66	1.97 ns	49.41	3.95 ns	625.69	0.76 ns	43.04	1.46 ns	1.30	0.15 ns	0.01	0.00 ns	2.52	0.16 ns
Sp × Zo	1	12.38	0.74 ns	28.17	0.75 ns	119.16	2.50 ns	148.83	1.73 ns	358.50	0.42 ns	182.72	5.91 ns	7.45	0.20 ns	0.02	0.00 ns	131.27	8.68 ns
Sp × Ar (Zo)	2	16.73	2.33 ns	37.41	6.54 **	47.71	3.03 ns	86.22	7.94 **	859.34	2.92 ns	30.90	3.04 ns	36.89	6.61 **	52.82	10.58 ***	15.13	4.11 *
Se × Zo	1	53.68	3.16 ns	8.26	1.51 ns	85.79	3.96 ns	10.19	2.56 ns	2102.25	318.94 **	6.21	3.68 ns	39.80	4.80 ns	23.31	15.26 ns	0.71	1.06 ns
Se × Ar (Zo)	2	16.96	2.36 ns	5.46	0.95 ns	21.64	1.38 ns	3.99	0.37 ns	6.59	0.02 ns	1.69	0.17 ns	8.30	1.49 ns	1.53	0.31 ns	0.67	0.18 ns
Sp × Se × Zo	1	0.70	0.15 ns	10.18	0.68 ns	5.69	0.20 ns	3.94	0.32 ns	1035.28	1.26 ns	1.43	0.05 ns	2.12	0.24 ns	3.42	0.21 ns	2.41	0.15 ns
Sp × Se × Ar (Zo)	2	4.60	0.64 ns	14.93	2.61 ns	28.76	1.83 ns	12.51	1.15 ns	821.63	2.80 ns	29.53	2.90 ns	8.81	1.58 ns	16.02	3.21 ns	15.96	4.33 *
Res	32	7.19		5.72		15.72		10.86		293.95		10.17		5.58		4.99		3.68	
Cochran's test		$C = 0.2510$ ns		$C = 0.2636$ ns		$C = 0.2744$ ns		$C = 0.2829$ ns		$C = 0.2476$ ns		$C = 0.1934$ ns		$C = 0.2130$ ns		$C = 0.2207$ ns		$C = 0.2673$ ns	
Transformation		(none)		(none)		(none)		(none)		(none)		(none)		(none)		(none)		(none)	
SNK tests				*Sex*		*Sex*		*Sex*		*Se × Zo*		*Species*		*Sp × Ar (Zo)*		*Sex*		*Sp × Se × Ar (Zo)*	

S.E. = 0.48	S.E. = 0.95	S.E. = 0.41	S.E. = 0.74	S.E. = 1.13	S.E. = 0.96	S.E. = 0.25	S.E. = 1.11
M < F*	M > F*	M > F*	Zo1, M < F**	Pu > Pd*	Zo1A1, Pu < Pd**	M > F**	M Zo1A1, Pu > Pd**
Sp×Ar (Zo)	*Zone*	*Sp×Ar (Zo)*	Zo2, F = M ns	*Sex*	Zo1A2, Pu < Pd*	*Sp×Ar (Zo)*	M Zo1A2, Pu > Pd**
S.E = 0.98	S.E. = 0.42	S.E. = 1.35	M, Zo1 = Zo2 ns	S.E. = 0.27	Zo2A1, Pu = Pd ns	S.E. = 0.91	M Zo2A1, Pu > Pd**
Zo1A1, Pu < Pd**	Zo1 > Zo2*	Zo1A1, Pu > Pd*	F, Zo1 > Zo2**	M > F*	Zo2A2, Pu < Pd**	Zo1A1, Pu < Pd**	M Zo2A2, Pu = Pd ns
Zo1A2, Pu < Pd**		Zo1A2, Pu > Pd**		Zone		Zo1A2, Pu = Pd ns	F Zo1A1, Pu > Pd**
Zo2A1, Pu < Pd**		Zo2A1, Pu > Pd*		S.E. = 0.30		Zo2A1, Pu = Pd ns	F Zo1A2, Pu > Pd**
Zo2A2, Pu = Pd ns		Zo2A2, Pu = Pd ns		Zo1 > Zo2**		Zo2A2, Pu < Pd**	F Zo2A1, Pu > Pd**
							F Zo2A2, Pu > Pd**
							Pu Zo1A1, M > F**
							Pu Zo1A2, M = F ns
							Pu Zo2A1, M = F ns
							Pu Zo2A2, M = F ns
							Pd Zo1A1, M = F ns
							Pd Zo1A2, M > F**
							Pd Zo2A1, M = F ns
							Pd Zo2A2, M > F*

ns = not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (M = male, F = female, Pu = *P. ulyssiponensis*, Pd = *P. depressa*, Zo1 = non-overlapping zone (upper mid-shore + low-shore); Zo2 = overlapping zone (lower mid-shore)).

Table 4
Fatty acid composition (% of total FAME) of the male and female *P. depressa* gonad, in the different stages of development, during summer and winter ($n=12$ per sex per stage II and III); in bold are the sum of the classes

Fatty acids % total FAME	Summer				Winter			
	Stage II		Stage III		Stage II		Stage III	
	Male	Female	Male	Female	Male	Female	Male	Female
13:0	0.12±0.06	0.10±0.01	0.13±0.02	0.11±0.02	0.02±0.01	0.02±0.01	0.00±0.00	0.01±0.01
14:0	4.58±0.39	8.83±0.27	4.09±0.42	8.87±0.13	5.32±0.44	8.17±0.75	4.61±0.20	8.50±0.37
15:0	1.06±0.07	0.74±0.05	1.04±0.09	0.76±0.03	1.30±0.08	0.97±0.11	1.37±0.07	0.98±0.04
16:0	20.57±1.39	30.47±0.97	17.82±1.68	30.27±0.83	23.86±0.92	33.52±0.76	24.36±1.17	33.49±0.68
17:0	0.79±0.10	0.37±0.01	0.44±0.00	0.46±0.01	0.54±0.03	0.45±0.04	0.40±0.03	0.46±0.06
18:0	7.09±0.14	3.90±0.07	7.10±0.22	3.93±0.08	6.89±0.33	3.11±0.18	6.57±0.07	3.10±0.11
20:0	0.04±0.03	0.04±0.01	0.09±0.03	0.24±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
22:0	0.08±0.04	0.07±0.04	0.15±0.10	0.05±0.02	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Σ SFA	**34.32±1.78**	**44.50±1.19**	**30.85±2.05**	**44.68±1.08**	**37.92±1.24**	**46.23±1.13**	**37.32±1.31**	**46.54±1.06**
14:1(n-5)	0.13±0.01	0.16±0.01	0.07±0.00	0.15±0.01	0.03±0.02	2.87±0.14	0.39±0.03	2.67±0.15
16:1(n-7)	1.67±0.64	5.01±0.26	0.84±0.14	4.25±0.35	2.16±0.14	5.74±0.28	1.86±0.24	6.19±0.29
16:1(n-5)	0.95±0.18	1.41±0.06	0.50±0.09	1.33±0.05	0.33±0.04	0.45±0.02	0.64±0.07	0.57±0.04
17:1(n-8)	0.38±0.06	0.04±0.01	0.27±0.04	0.05±0.02	0.32±0.02	0.15±0.01	0.35±0.02	0.25±0.01
18:1(n-9)	6.99±0.69	8.33±0.14	5.39±0.35	8.23±0.14	5.33±0.35	8.02±0.25	4.87±0.21	8.01±0.13
18:1(n-7)	14.98±0.45	17.81±0.31	15.01±0.43	17.52±0.44	15.59±0.41	20.10±0.58	16.40±0.16	19.83±0.55
18:1(n-5)	0.08±0.02	0.00±0.00	0.08±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
20:1(n-9)	4.05±1.63	5.02±0.60	4.52±0.34	5.15±0.17	3.56±0.27	3.12±0.44	3.07±0.16	2.62±0.51
20:1(n-7)	2.43±0.30	3.35±0.51	2.17±0.17	3.53±0.23	1.83±0.31	4.27±0.38	1.97±0.09	4.19±0.56
20:1(n-5)	0.56±0.14	0.22±0.07	0.68±0.07	0.29±0.06	0.19±0.12	0.00±0.00	0.15±0.01	0.00±0.00
22:1(n-11)	0.34±0.19	0.02±0.02	0.17±0.10	0.06±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
22:1(n-9)	0.09±0.06	1.18±0.18	0.35±0.21	0.98±0.12	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
24:1(n-9)	0.00±0.00	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
24:1(n-7)	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Σ MUFA	**32.63±1.79**	**42.54±1.02**	**30.05±0.87**	**41.57±1.06**	**29.32±0.70**	**44.69±0.89**	**29.68±0.37**	**44.32±0.77**
Iso 15:0	0.25±0.03	0.08±0.03	0.22±0.04	0.10±0.04	0.02±0.02	0.00±0.00	0.06±0.01	0.02±0.00
Anteiso 15:0	0.01±0.01	0.00±0.00	0.02±0.01	0.01±0.01	0.04±0.02	0.02±0.01	0.01±0.01	0.02±0.00
Iso 16:0	0.95±0.11	0.98±0.06	0.88±0.09	0.99±0.03	1.14±0.06	0.69±0.04	1.18±0.06	0.93±0.04
Anteiso 16:0	0.68±0.11	0.07±0.01	0.66±0.12	0.05±0.01	0.63±0.04	0.24±0.03	0.67±0.04	0.01±0.01
Iso 17:0	1.00±0.11	0.95±0.06	0.74±0.12	0.86±0.07	0.80±0.09	0.76±0.04	0.83±0.07	0.81±0.03
Anteiso 17:0	0.32±0.06	0.00±0.00	0.48±0.07	0.00±0.00	0.30±0.03	0.76±0.05	0.23±0.01	0.75±0.08
Iso 18:0	1.35±0.15	0.77±0.11	1.38±0.32	0.70±0.08	1.56±0.07	0.75±0.05	1.26±0.07	0.63±0.03
Σ Branched	**4.55±0.28**	**2.85±0.17**	**4.37±0.35**	**2.71±0.08**	**4.48±0.18**	**3.22±0.15**	**4.15±0.15**	**3.17±0.11**
16:3(n-4)	0.58±0.24	0.39±0.03	0.72±0.14	0.39±0.05	0.09±0.05	0.34±0.02	0.38±0.01	0.31±0.02
16:3(n-3)	0.92±0.07	0.11±0.05	0.60±0.11	0.15±0.01	0.02±0.02	0.00±0.00	0.72±0.05	0.00±0.00
16:4(n-3)	0.32±0.04	0.38±0.02	0.37±0.03	0.40±0.02	0.80±0.08	0.54±0.04	1.22±0.05	0.66±0.04
16:4(n-1)	0.41±0.15	0.12±0.03	0.09±0.04	0.15±0.03	2.10±0.20	0.65±0.03	1.16±0.05	0.40±0.03

18:2(n-6)	0.94 ± 0.07	0.62 ± 0.04	0.73 ± 0.06	0.57 ± 0.03	0.93 ± 0.16	0.42 ± 0.04	0.84 ± 0.05	0.63 ± 0.04
18:3(n-3)	0.48 ± 0.08	0.31 ± 0.08	0.50 ± 0.09	0.23 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:4(n-3)	0.12 ± 0.06	0.11 ± 0.03	0.17 ± 0.04	0.11 ± 0.03	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:2(n-6)	0.91 ± 0.12	0.88 ± 0.09	0.96 ± 0.08	1.10 ± 0.08	1.43 ± 0.17	0.44 ± 0.13	1.07 ± 0.27	0.54 ± 0.16
20:3(n-6)	0.44 ± 0.05	0.37 ± 0.04	0.29 ± 0.02	0.47 ± 0.07	0.29 ± 0.04	0.00 ± 0.00	0.88 ± 0.11	0.12 ± 0.06
20:4(n-6)	8.38 ± 0.82	3.46 ± 0.24	9.02 ± 0.56	4.40 ± 0.57	5.08 ± 0.35	1.46 ± 0.17	4.88 ± 0.21	1.38 ± 0.17
20:3(n-3)	1.24 ± 0.19	0.63 ± 0.12	1.77 ± 0.14	0.71 ± 0.09	0.00 ± 0.00	0.17 ± 0.10	0.94 ± 0.51	0.09 ± 0.05
20:4(n-3)	0.26 ± 0.05	0.28 ± 0.09	0.39 ± 0.11	0.27 ± 0.06	1.25 ± 0.22	0.01 ± 0.01	0.47 ± 0.15	0.07 ± 0.04
20:5(n-3)	12.27 ± 1.45	1.11 ± 0.12	17.84 ± 1.31	1.13 ± 0.19	16.07 ± 0.88	1.64 ± 0.20	16.84 ± 1.01	1.78 ± 0.38
21:5(n-3)	0.03 ± 0.03	0.72 ± 0.19	0.25 ± 0.09	0.23 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22:4(n-6)	0.05 ± 0.02	0.24 ± 0.14	0.13 ± 0.04	0.30 ± 0.07	0.09 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22:5(n-6)	0.02 ± 0.02	0.02 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22:5(n-3)	0.15 ± 0.06	0.06 ± 0.04	0.18 ± 0.07	0.06 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22:6(n-3)	1.01 ± 0.23	0.32 ± 0.09	0.67 ± 0.08	0.34 ± 0.07	0.12 ± 0.07	0.18 ± 0.03	0.11 ± 0.01	0.01 ± 0.01
Σ PUFA	**28.50 ± 2.26**	**10.11 ± 0.56**	**34.73 ± 2.19**	**11.05 ± 0.95**	**28.28 ± 1.65**	**5.86 ± 0.43**	**28.86 ± 1.20**	**5.98 ± 0.40**
Σ HUFA	**24.74 ± 2.22**	**8.09 ± 0.49**	**31.56 ± 2.08**	**9.05 ± 0.91**	**24.33 ± 1.53**	**3.90 ± 0.40**	**24.54 ± 1.19**	**3.97 ± 0.45**
Σ(n-3)	**16.78 ± 1.56**	**4.02 ± 0.52**	**22.75 ± 1.69**	**3.62 ± 0.39**	**18.27 ± 1.14**	**2.55 ± 0.28**	**20.30 ± 1.24**	**2.60 ± 0.33**
Σ(n-6)	**10.74 ± 0.85**	**5.58 ± 0.41**	**11.18 ± 0.67**	**6.88 ± 0.68**	**7.82 ± 0.60**	**2.31 ± 0.18**	**7.02 ± 0.48**	**2.66 ± 0.41**
Σ(n-3)/Σ(n-6)	**1.75 ± 0.11**	**0.75 ± 0.10**	**2.18 ± 0.12**	**0.56 ± 0.05**	**2.45 ± 0.13**	**1.31 ± 0.14**	**3.17 ± 0.29**	**1.03 ± 0.19**
DHA/EPA	0.11 ± 0.04	0.29 ± 0.06	0.04 ± 0.01	0.32 ± 0.08	0.01 ± 0.01	0.10 ± 0.03	0.01 ± 0.00	0.00 ± 0.00
Σ Total FAME (μg/mg DW)	**44.54 ± 3.22**	**149.44 ± 4.81**	**36.65 ± 2.02**	**140.59 ± 5.25**	**34.86 ± 0.90**	**113.30 ± 7.30**	**33.49 ± 1.19**	**112.85 ± 3.75**

Data are means ± S.E.

Table 5
Analysis of variance on the fatty acid composition (% of total FAME) of *P. depressa* stage II gonad

Source of variation	d.f.	SFA		MUFA		PUFA		HUFA		FAME		(n-3)		(n-6)		ARA		EPA	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Sex = Se	1	1026.20	11.19 ns	1917.99	210.08 **	3041.85	38.80 *	27.16	47.80 *	100 848.00	70.63 *	34.24	81.40 *	341.39	264.23 **	441.75	406.65 **	66.57	67.57 *
Season = Sa	1	85.49	5.53 ns	3.97	0.05 ns	69.14	1.81 ns	1.92	15.76 ns	6297.96	4.05 ns	0.45	4.04 ns	115.04	3.51 ns	167.28	4.23 ns	1.53	5.70 ns
Date = Da (Sa)	1	15.45	0.47 ns	74.40	2.48 ns	38.15	5.13 ns	0.12	1.72 ns	1553.97	4.31 ns	0.11	1.17 ns	32.77	4.69 ns	39.51	4.85 ns	0.27	1.12 ns
Area = Ar (Da) (Sa)	4	32.55	4.33 **	29.96	5.47 **	7.43	2.06 ns	0.07	2.37 ns	360.75	4.32 **	0.09	2.37 ns	6.99	5.52 **	8.15	7.46 ***	0.24	5.33 **
Se × Sa	1	10.40	0.11 ns	89.27	9.78 ns	54.23	0.69 ns	1.57	2.77 ns	2099.60	1.47 ns	0.84	1.99 ns	0.39	0.30 ns	0.00	0.00 ns	0.12	0.12 ns
Se × Da	1	91.67	0.92 ns	9.12	0.23 ns	74.41	5.63 *	0.57	2.46 ns	1427.85	3.16 ns	0.42	2.35 ns	1.29	0.16 ns	1.08	0.11 ns	0.99	7.83 *
Se × Ar (Da) (Sa)	4	99.41	13.22 ***	39.13	7.14 ***	13.92	3.85 *	0.23	7.73 ***	452.23	5.42 **	0.18	4.51 **	8.28	6.54 ***	10.34	9.46 ***	0.13	2.80 *
Res	32	7.52		5.48		3.62		0.03		83.50		0.04		1.26		1.09		0.05	
Cochran's test		*C* = 0.3204 ns		*C* = 0.2264 ns		*C* = 0.3319 ns		*C* = 0.2413 ns		*C* = 0.2960 ns		*C* = 0.2933 ns		*C* = 0.2799 ns		*C* = 0.3160 ns		*C* = 0.3221 ns	
Transformation		(none)		(none)		(ArcSin)		(Ln)		(none)		(Ln)		(none)		(ArcSin)		(Ln)	
SNK tests		*Se×Ar (Da) (Sa)* S.E. = 1.58 SDa1Ar1, M<F** SDa1Ar2, M<F** SDa2Ar1, M<F** SDa2Ar2, M<F** WDa1Ar1, M=F ns WDa1Ar2, M<F* WDa2Ar1, M=F ns WDa2Ar2, M<F**		*Se×Ar (Da) (Sa)* S.E. = 1.35 SDa1Ar1, M<F* SDa1Ar2, M<F** SDa2Ar1, M<F** SDa2Ar2, M<F** WDa1Ar1, M<F** WDa1Ar2, M<F** WDa2Ar1, M<F** WDa2Ar2, M<F**		*Se×Ar (Da) (Sa)* S.E. = 1.10 SDa1Ar1, M>F** SDa1Ar2, M>F** SDa2Ar1, M>F** SDa2Ar2, M>F** WDa1Ar1, M>F** WDa1Ar2, M>F** WDa2Ar1, M>F** WDa2Ar2, M>F**		*Se×Ar (Da) (Sa)* S.E. = 0.10 SDa1Ar1, M>F** SDa1Ar2, M>F** SDa2Ar1, M>F** SDa2Ar2, M>F** WDa1Ar1, M>F** WDa1Ar2, M>F** WDa2Ar1, M>F** WDa2Ar2, M>F**		*Se×Ar (Da) (Sa)* S.E. = 5.28 SDa1Ar1, M<F** SDa1Ar2, M<F** SDa2Ar1, M<F** SDa2Ar2, M<F** WDa1Ar1, M<F** WDa1Ar2, M<F** WDa2Ar1, M<F** WDa2Ar2, M<F**		*Se×Ar (Da) (Sa)* S.E. = 0.12 SDa1Ar1, M>F** SDa1Ar2, M>F** SDa2Ar1, M>F** SDa2Ar2, M>F** WDa1Ar1, M>F** WDa1Ar2, M>F** WDa2Ar1, M>F** WDa2Ar2, M>F**		*Se×Ar (Da) (Sa)* S.E. = 0.65 SDa1Ar1, M>F** SDa1Ar2, M>F** SDa2Ar1, M>F** SDa2Ar2, M>F** WDa1Ar1, M>F** WDa1Ar2, M>F** WDa2Ar1, M>F** WDa2Ar2, M>F**		*Se×Ar (Da) (Sa)* S.E. = 0.60 SDa1Ar1, M>F** SDa1Ar2, M>F** SDa2Ar1, M>F** SDa2Ar2, M>F** WDa1Ar1, M>F** WDa1Ar2, M>F** WDa2Ar1, M>F** WDa2Ar2, M>F**		*Se×Ar (Da) (Sa)* S.E. = 0.12 SDa1Ar1, M>F** SDa1Ar2, M>F** SDa2Ar1, M>F** SDa2Ar2, M>F** WDa1Ar1, M>F** WDa1Ar2, M>F** WDa2Ar1, M>F** WDa2Ar2, M>F**	

ns = not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (M = male, F = female, S = summer, W = winter).

Table 6
Analysis of variance on the fatty acid composition (% of total FAME) of *P. depressa* stage III gonad

Source of variation	d.f.	SFA		MUFA		PUFA		HUFA		FAME		(n-3)		(n-6)		ARA		EPA	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Sex = Se	1	1593.22	209.28 **	734.99	54.75 *	6504.43	241.86 **	5567.23	269.14 **	19.72	491.97 ***	4068.61	112.52 **	228.94	16.60 ns	198.37	28.88 *	3022.76	61.74 *
Season = Sa	1	208.08	5.36 ns	5.45	2.14 ns	359.49	2.80 ns	439.59	5.34 ns	0.27	1.61 ns	35.98	0.47 ns	206.38	7.34 ns	154.23	10.45 ns	0.30	0.00 ns
Date = Da (Sa)	1	38.79	0.41 ns	2.55	0.46 ns	128.54	1.29 ns	82.26	0.88 ns	0.17	5.99 ns	77.10	3.03 ns	28.10	1.16 ns	14.76	0.81 ns	88.00	4.42 ns
Area = Ar (Da) (Sa)	4	94.36	13.31 ***	5.50	5.57 **	99.74	15.63 ***	93.38	15.41 ***	0.03	4.14 **	25.44	6.50 ***	24.21	18.90 ***	18.30	28.31 ***	19.89	8.06 ***
Se × Sa	1	63.85	8.39 ns	10.04	0.75 ns	1.93	0.07 ns	11.31	0.55 ns	0.05	80.81 *	6.02	0.17 ns	0.06	0.00 ns	3.70	0.54 ns	8.56	0.17 ns
Se × Da	1	7.61	0.17 ns	13.42	1.70 ns	26.89	4.29 ns	20.69	2.15 ns	0.00	0.07 ns	36.16	3.14 ns	13.78	2.54 ns	6.87	1.48 ns	48.96	3.39 ns
Se × Ar (Da) (Sa)	4	44.76	6.32 ***	7.90	8.01 ***	6.27	0.98 ns	9.62	1.59 ns	0.01	1.37 ns	11.50	2.94 *	5.43	4.24 **	4.66	7.20 ***	14.42	5.84 **
Res	32	7.09		0.99		6.38		6.06		0.01		3.91		1.28		0.65		2.47	
Cochran's test		*C* = 0.1583 ns		*C* = 0.4921 (P < 0.01)		*C* = 0.2331 ns		*C* = 0.2186 ns		*C* = 0.2470 ns		*C* = 0.2071 ns		*C* = 0.1788 ns		*C* = 0.2653 ns		*C* = 0.2811 ns	
Transformation		(none)		(ArcSin)		(none)		(none)		(Ln)		(none)		(none)		(none)		(none)	
SNK tests		*Se × Ar (Da) (Sa)* S.E. = 1.54 SDa1Ar1, M < F** SDa1Ar2, M < F** SDa2Ar1, M < F** SDa2Ar2, M < F** WDa1Ar1, M < F** WDa1Ar2, M < F** WDa2Ar1, M = F ns WDa2Ar2, M < F**		*Se × Ar (Da) (Sa)* S.E. = 0.57 SDa1Ar1, M < F** SDa1Ar2, M < F** SDa2Ar1, M < F** SDa2Ar2, M < F** WDa1Ar1, M < F** WDa1Ar2, M < F** WDa2Ar1, M < F** WDa2Ar2, M < F**		*Se* S.E. = 1.06 M > F**		*Se* S.E. = 0.93 M > F**		*Se × Sa* S.E. = 0.01 S, M < F** W, M < F** M, S > W* F, S > W**		*Se × Ar (Da) (Sa)* S.E. = 1.14 SDa1Ar1, M > F** SDa1Ar2, M > F** SDa2Ar1, M > F** SDa2Ar2, M > F** WDa1Ar1, M > F** WDa1Ar2, M > F** WDa2Ar1, M > F** WDa2Ar2, M > F**		*Se × Ar (Da) (Sa)* S.E. = 0.65 SDa1Ar1, M = F ns SDa1Ar2, M > F** SDa2Ar1, M > F** SDa2Ar2, M > F** WDa1Ar1, M > F** WDa1Ar2, M > F** WDa2Ar1, M > F** WDa2Ar2, M = F ns		*Se × Ar (Da) (Sa)* S.E. = 0.46 SDa1Ar1, M > F** SDa1Ar2, M > F** SDa2Ar1, M > F** SDa2Ar2, M > F** WDa1Ar1, M > F** WDa1Ar2, M > F** WDa2Ar1, M > F** WDa2Ar2, M > F**		*Se × Ar (Da) (Sa)* S.E. = 0.91 SDa1Ar1, M > F** SDa1Ar2, M > F** SDa2Ar1, M > F** SDa2Ar2, M > F** WDa1Ar1, M > F** WDa1Ar2, M > F** WDa2Ar1, M > F** WDa2Ar2, M > F**	

ns = not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (M = male, F = female; S = summer, W = winter).

cantly higher amounts of SFA, MUFA (given that variances were heterogeneous in stage III, these significant differences should be regarded with some caution) and total FAME while in males significantly higher percentages were found for ARA, EPA as well as for total PUFA, HUFA and fatty acids from the (n-3) and (n-6) series (Tables 5 and 6 and Fig. 1). An interaction between sex and area was found in all these fatty acid classes, with the exception of PUFA and HUFA in stage III (Tables 5 and 6, SNK tests). However, the comparison between seasons only revealed significant differences for total FAME in stage III (where an interaction between sex and season was found) with a significantly higher amount of total FAME in summer than in winter gonads (Table 6). Large amounts of the SFA, especially 16:0 were also found in the gonads of both sexes, during summer and winter (Table 4).

4. Discussion

The lipid levels in the body components of marine invertebrates tend to vary considerably, with the physical environment, the dietary state of the organisms and the annual reproductive cycle being the most likely sources of variation (Simpson, 1982). The major fatty acids found in the soft bodies of all four species of *Patella* spp. (*P. depressa*, *P. ulyssiponensis*, *P. vulgata* and *P. rustica*) were the SFA 14:0, 16:0, 18:0, the MUFA 16:1(n-7), 18:1(n-7), 18:1(n-9), 20:1(n-9), and the PUFA ARA and EPA. This fatty acid profile is very similar to that found in previous studies concerning prosobranch gastropods (Dunstan et al., 1996).

The PUFA EPA and DHA have been shown to be essential fatty acids and important tissue components in a variety of molluscs, particularly bivalves (Pastoriza et al., 1980, 1981; Beninger and Stephan, 1985; Kluytmans et al., 1985; Piretti et al., 1987, 1988; Wenne and Polak, 1989; Chu et al., 1990; Hayashi and Kishimura, 1991; Abad et al., 1995; Pazos et al., 1996, 1997; Galap et al., 1999). However, in *Patella* spp. soft bodies and *P. depressa* gonads, DHA was present in very low amounts, while large percentages of EPA and ARA were found. This result suggests that *Patella* spp. probably have different dietary requirements for essential fatty acids (namely DHA), a pattern that has already been described in *Haliotis* species (Dunstan et al., 1996).

Palmitic acid (16:0) was the most abundant saturated fatty acid found in all four limpets. According to Gabbott (1983), this fatty acid is the major end product of the fatty acid synthetase system in animal tissues and is the precursor for de novo synthesis of long-chain saturated and unsaturated fatty acids. In addition, Brown et al. (1997) pointed out that diets richer in saturated fatty acids are more nutritious for rapidly growing larvae, because energy is released more efficiently from saturated fats than from unsaturated fats. This observation supports the higher 16:0 and SFA levels (and, consequently, the higher total FAME content) found in the gonads, but less obvious in the soft bodies of female limpets, since female gametes have the important role of providing the energy reserves for the developing embryo (Blackmore, 1969; Sargent et al., 1989).

The quality as well as the quantity of algal lipids is very important in the nutrition of marine animals as algae are main sources of the essential fatty acids (the PUFA, ARA, EPA and DHA) that marine molluscs cannot synthesise de novo (Sukenik et al., 1993; Abad et al., 1995; Brown et al., 1997). Diatoms (Bacillariophycea) are rich sources of EPA, ARA and to a lesser extent of C16 PUFA. Dinoflagellates (Dinophycea) are richer in DHA. Green algae (Chlorophycea) tend to be rich in C16 and C18 (n-3) PUFA, especially 18:4(n-3) and deficient in both C20 and C22 PUFAs. Red algae (Rhodophyta), besides being rich in (n-3) PUFA, mainly EPA, may have considerable amounts of ARA. Finally, brown algae tend to be rich in ARA and EPA but significant levels of C18 (n-3) PUFA are also found. In all the groups of macroalgae, C22 PUFA is not present in high levels (Chuecas and Riley, 1969; Sargent et al., 1989; Dunstan et al., 1996; Brown et al., 1997). According to Dunstan et al. (1996), who worked with *Haliotis* spp., the elevated content of EPA instead of DHA in herbivorous species is possibly an adaptation to a low lipid macroalgal diet. This might explain the low level of DHA found in the *Patella* spp. soft body and gonads.

Another interesting result was the difference found between *P. ulyssiponensis* and *P. depressa* soft bodies. *P. ulyssiponensis* showed significantly higher percentages of EPA, HUFA and (n-3) fatty acids, while elevated proportions of ARA, MUFA and (n-6) fatty acids were found in *P. depressa*. However, this pattern was not consistently found in all areas. The fatty acid profiles are probably linked with the limpet's diet and position on the shore (which will ultimately affect their diet). *P. ulyssiponensis* occurs in the low-shore level where a large variety of different algae can be found, such as red algal turf and encrusting algae rich in (n-3) PUFA, mainly EPA. However, *P. depressa* inhabits the mid-shore zone that has a lower availability of macroalgae and is colonised by microbial films and ephemeral algae, particularly greens. Position on the shore and potential dietary differences are probably responsible for the significant differences found between both species. In the overlapping zone, where both species coexist, fewer significant differences were found among them. This result emphasizes the idea that diet is an important factor in the fatty acid profile of these species.

In the present study, an attempt was also made to compare the gonad fatty acid profile in *P. depressa* during winter and summer, and in different stages of gonad development. The statistical analysis revealed significant differences between male and female gonads, while differences between seasons were not so evident. Thus, female gonads showed significantly higher amounts of SFA, MUFA and FAME whereas in males significantly higher percentages were found for PUFA, HUFA, ARA, EPA and fatty acids from the (n-3) and (n-6) series (as a consequence of the high percentage of EPA and ARA, respectively). These differences are not unexpected since the male gametes are known to be composed mostly of structural polar lipids rich in PUFA and HUFA, while in female gonads SFA and MUFA are important energy sources that will be transferred to the developing embryo (Blackmore, 1969). In *P. vulgata*, Blackmore (1969) found that in both sexes lipids are accumulated in the gonad during maturation and that a higher lipid content was found in the female gonad. Therefore, the higher amount of total FAME found in the female *P. depressa* gonads confirms the results obtained for *P. vulgata*. These results were already suggested by the analysis of the female and male whole soft body of several species of *Patella*, although some of the differences were not as clear as in the gonads due to the contribution of other organs, such as the digestive gland and the mantle, that largely determine the fatty acid composition of the whole body.

In conclusion, the results obtained in the present work reveal that the quantitatively most important fatty acids are quite similar in the four *Patella* species analysed, and that the fatty acid profiles of these limpets conform to the common pattern characteristic of gastropods in general. In addition, the results indicate that the environmental conditions to which the limpets are submitted, in particular the diet, affect their lipid composition, although a detailed characterization of the areas from which limpets were collected would allow a more comprehensive analysis. It is also shown that *P. depressa* female gonads are richer in SFA and MUFA (typical of neutral lipids), whose function is mainly the provision of metabolic energy, while male gonads are mostly composed of PUFA-rich structural lipids. The analysis of the whole soft body fatty acid composition of the four *Patella* species suggests that the same might be true in other limpet species.

Acknowledgments

We would like to thank Ana Silva for all the given support in the field work and also to Milton Alfredo for helping with the laboratory work.

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. C 110, 109–118.

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

Ben-Amotz, A., Fishler, R., Schneller, A., 1987. Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. Mar. Biol. 95, 31–36.

Beninger, P.G., Stephan, G., 1985. Seasonal variations in the fatty acids of the triacylglycerols and phospholipids of two populations of adult clam (*Tapes decussates* L. and *T. philippinarum*) reared in a common habitat. Comp. Biochem. Physiol. B 81, 591–601.

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

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

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

Branch, G.M., 1981. The biology of limpets: physical factors, energy flow and ecological interactions. Oceanogr. Mar. Biol. Annu. Rev. 19, 235–380.

Brown, M.R., Jeffrey, S.W., Volkman, J.K, Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151, 315–331.

Christie, W.W., 1982. Lipid Analysis. 2nd ed. Pergamon Press, Oxford.

Chu, F.L., Webb, K.L., Chen, J., 1988. Seasonal changes of lipids and fatty acids in oysters. J. Shellfish Res. 7, 209.

Chu, F.L., Webb, K.L., Chen, J., 1990. Seasonal changes of lipids and fatty acids in oyster tissues (*Crassostrea virginica*) and estuarine particulate matter. Comp. Biochem. Physiol. A 95, 385–391.

Chuecas, L., Riley, J.P., 1969. Component fatty acids of the total lipids of some marine phytoplankton. J. Mar. Biol. Ass. UK 49, 97–116.

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.

Fretter, V., Graham, A., 1962. British Prosobranch Molluscs. Ray Society, London.

Fretter, V., Graham, A., 1976. The prosobranch mollucs of Britain and Denmark. I. Pleurotomariacea, Fissurellacea and Patellacea. J. Moll. Stud. (Suppl. 1) 1–37.

Gabbott, P.A., 1983. Developmental and seasonal metabolic activities in marine molluscs. In: Hochachka, P.W. (Ed.), The Mollusca, 2, Environmental Biochemistry and Physiology. Academic Press, New York, pp. 165–217.

Gabbott, P.A., Bayne, B.L., 1973. Biochemical effects of temperature and nutritive stress on *Mytilus edulis* L. J. Mar. Biol. Ass. UK 53, 269–286.

Galap, C., Netchitailo, P., Leboulenger, F, Grillot, J., 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. A 112, 241–254.

Gardner, D., Riley, J.P., 1972. The component fatty acids of the lipids of some species of marine and freshwater mollucsc. J. Mar. Biol. Ass. UK 52, 827–838.

Hayashi, K., Kishimura, H., 1991. Seasonal changes in the contents of eicosapentaenoic acid-containing triaglycerides in hepatopancreas of scallop. Nippon Suisan Gakkaishi 57 (7), 1397–1401.

Kluytmans, J.H., Boot, J.H., Oudejans, R.C.H.M., 1985. Fatty acid synthesis in relation to gametogenesis in the mussel *Mytilus edulis* L. Comp. Biochem. Physiol. B 81, 959–963.

Metcalfe, L.D., Schmitz, A.A., 1961. The rapid preparation of fatty acid esters for gas chromatography analysis. Anal Chem. 33, 363–364.

Narciso, L., 1996. Biologia e cultivo de alguns crustáceos marinhos, com especial ênfase para as fases larvares de *Artemia* sp. (Branchiopoda), *Penaeus kerathurus* e *Palaemon serratus* (Decapoda). Ph.D. Thesis, Portugal: Faculdade de Ciências da Universidade de Lisboa.

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. Ass. UK 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. Ass. UK 35, 149–176.

Pastoriza, L., Gallardo, J.M., Franco, J.M., Sampedro, G., 1980. Ácidos grasos de la vieira (*Pecten maximus*). Inv. Pesq. 44, 505–510.

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., Ruiz, C., Garcia-Martin, O., Abad, M., Sanchez, 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. B 114, 171–179.

Pazos, A.J., Romàn, 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. B 117, 393–402.

Piretti, M.V., Taioli, F., Pagliuca, G., 1987. Investigation of the seasonal variations of sterol and fatty acid constituents in the bivalve molluscs *Venus gallina* and *Scapharca inaequivalvis* (Bruguiére). Comp. Biochem. Physiol. B 88, 1201–1208.

Piretti, M.V., Zuppa, F., Pagliuca, G., Taioli, F., 1988. Investigation of the seasonal variations of the fatty acid constituents in selected tissues of the bivalve mollusc *Scapharca inaequivalvis* (Bruguiere). Comp. Biochem. Physiol. B 89, 183–187.

Ruiz, C., Abad, M., Sedano, F., Garcia-Martin, L.O., 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., Hederson, R.J., Tocher, D.R., 1989. The lipids. In: Halver, J.E. (Ed.), Fish Nutrition. 2nd ed. Academic Press, London.

Sargent, J.R., 1995. Origins and functions of egg lipids: nutritional implications. In: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science Ltd, pp. 353–372.

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.

Sukenik, A., Zmora, O., Carmeli, Y., 1993. Biochemical quality of marine unicellular algae with special emphasis on lipid composition. II. *Nannochloropsis* sp. Aquaculture 117, 313–326.

Taylor, A.C., Venn, T.J., 1979. Seasonal variation in weight and biochemical composition of the tissues of the queen scallop, *Chlamys opercularis*, from the Clyde Sea area. J. Mar. Biol. Ass. UK 59, 605–621.

Underwood, A.J., 1997. Experiments in Ecology. Their logical Design and Interpretation using Analysis of Variance. Cambridge University Press, Cambridge.

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.

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