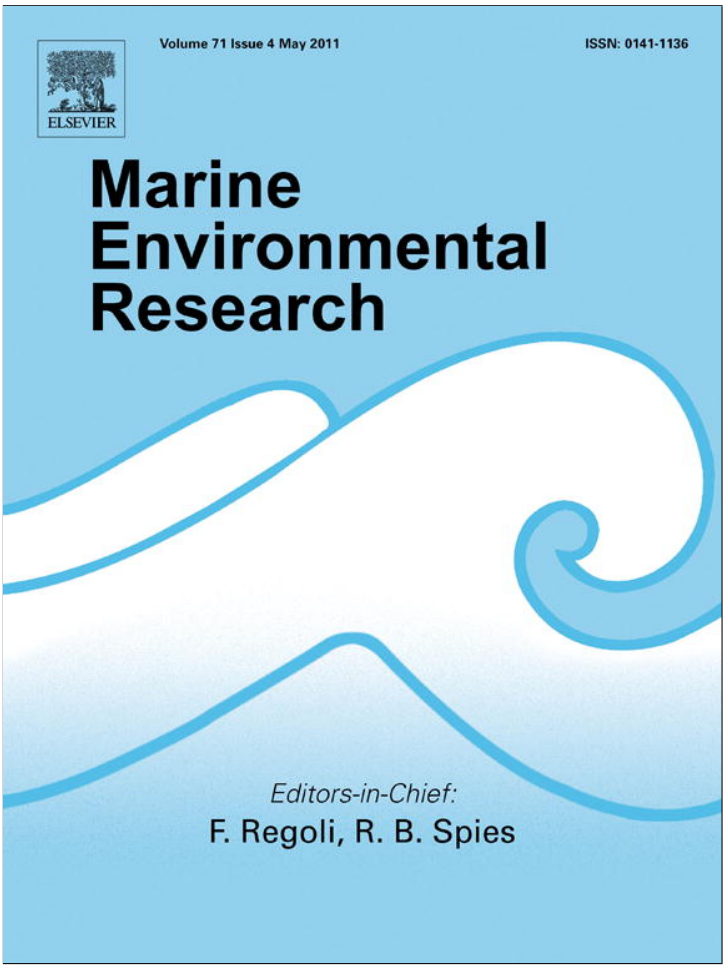

Marine Environmental Research 71 (2011) 235–246

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Trophic ecology of the sea urchin *Spatangus purpureus* elucidated from gonad fatty acids composition analysis

C. Barberá [a,*], D. Fernández-Jover [b], J.A. López Jiménez [c], D. González Silvera [c], H. Hinz [d], J. Moranta [a]

[a] Spanish Institute of Oceanography, Moll de Ponent s/n, 07015 Palma, Spain
[b] Departament of Marine Sciences, University of Alicante, P.O. Box 99, E-03080 Alicante, Spain
[c] Departament of Physiology, University of Murcia, P.O. Box 30100, Murcia, Spain
[d] School of Ocean Sciences, University of Wales, Bangor, United Kingdom

ARTICLE INFO

Article history:
Received 12 November 2010
Received in revised form
18 January 2011
Accepted 24 January 2011

Keywords:
Sea urchins
Fatty acids
Feeding
Diet
Soft red algae
Sandy bottoms
Sediment
Detritus
Bioturbation
Deposit feeder
Mediterranean sea

ABSTRACT

Irregular sea urchins such as the spatangoid *Spatangus purpureus* are important bioturbators that contribute to natural biogenic disturbance and the functioning of biogeochemical cycles in soft sediments. In the coastal waters of the Balearic Islands *S. purpureus* occurs in soft red algal beds, and can reach high densities. The diet of *S. purpureus* is unknown and it is particularly difficult to analyze the stomach contents of this group; therefore, we analyzed the fatty acid (FA) composition of the gonads and potential food resources in order to assess the trophic relationships of this species. The FA profiles of the gonads of *S. purpureus* agree well with the FA composition of the potential trophic resources (algae and sediment) and reveals changes between localities with different available resources. Three polyunsaturated FAs mainly contributes in the composition in the *S. purpureus* gonads: eicosapentaenoic acid (C20:5*n*-3) and arachidonic acid (C20:4*n*-6), both abundant in the macroalgal material, and palmitoleic acid (C16:1*n*-7), which is characteristic of sediment samples. Trophic markers of bacterial input and carnivorous feeding were significantly more abundant in sea urchins caught on bottoms with less vegetation. The current study demonstrates that the FA content of *S. purpureus* gonads is a useful marker of diet, as differences in the profiles reflected the variations in detritus composition. The results of this study show that this species has omnivorous feeding behavior; however, viewed in conjunction with available abundance data the results suggest that phytodetritus found within algal beds is an important carbon source for this species.

1. Introduction

Sea urchins are an important benthic megafaunal group, and play a significant ecologic role in the community structure. Regular sea urchins are generally herbivores and feed on micro- and macroalgae (Lawrence, 1975, 2007; Jangoux and Lawrence, 1982; Verlaque, 1987; Carpenter, 1981), while urchins with a burrowing life trait are generally considered detritivorous deposit feeders (Lawrence, 2007). Irregular urchins are common in subtidal soft sediments around the world, and play an important role in the biogenic disturbance (bioturbation) and biogeochemistry cycles in soft sediment systems (Ghiold, 1989; Widdicombe and Austen, 1999; Widdicombe et al., 2004; Lawrence, 2007; Lohrer et al., 2004, 2005, 2008). Large burrowing species such as spatangoids (heart urchins) are particularly important for these processes due to their abundance, size and mobility (Chiold, 1989). Large-scale losses of benthic bioturbators due to fishing disturbances could impair the functioning of marine ecosystems (Thrush and Dayton, 2002). Despite the obvious ecological importance of burrowing urchins, relatively little is known about the ecology of individual species, including their precise dietary requirements (Lawrence, 2007; Jangoux and Lawrence, 1982). This is due to the difficulties involved in traditional methods of stomach content analysis in which ingested material is often unidentifiable due to digestion processes. Fatty acids have recently been advocated as qualitative markers for tracing or confirming predator–prey relationships in the marine environment (Grahl-Nielsen et al., 2003; Iverson et al., 2004; Budge et al., 2006), identifying key processes in the dynamics of pelagic ecosystems (Brett and Müller-Navarra, 1997; Dalsgaard et al., 2003; Käkelä et al., 2005; Fernandez-Jover et al., 2007), and examining trophic interactions within benthic ecosystems (Ginger et al., 2000; Budge et al., 2002). The principle of this method is relatively simple. Consumers derive their lipid requirements either

* Corresponding author. Tel.: +34 971401877; fax: +34 971404945.
E-mail address: carmen.barbera@ba.ieo.es (C. Barberá).

0141-1136/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marenvres.2011.01.008

from their diet or by endogenous lipogenesis from dietary protein and carbohydrate precursors. Dietary lipids are broken down into their constituent FAs and are incorporated relatively unchanged into the tissues of the consumer (Lee et al., 1971; Stryer, 1995). Animals receive a considerable amount of lipid via their diet, and thus the diet type alters the FA composition of the organism (e.g. Sargent and Whittle, 1981; Sargent et al., 1987; Hughes et al., 2005; Hyne et al., 2009). Some polyunsaturated fatty acids (PUFAs) are considered essential. These are FAs that are necessary but cannot be synthesized by the organism, and thus must be consumed in the diet (Lenningher, 1984). Certain FAs, or their ratios, have specific known sources and can therefore act as "trophic markers", providing a more precise indication of an organism's diet than gut content analysis (Sargent et al., 1987; Dalsgaard et al., 2003; Howell et al., 2003). In addition, the sea urchins' diet varies locally and depends on food availability (Vadas, 1977; Ayling, 1978; Beddingfield and Mc Clintock, 1999). Diet quality has also been found to influence somatic and gonadal growth and development in urchins (Emson and Moore, 1998; Cook et al., 2000; Liyana-Pathiranaa et al., 2002; Liu et al., 2007a,b), and probably fecundity, as has been demonstrated in crustaceans (Hyne et al., 2009).

The spatangoid *Spatangus purpureus* (Müller, 1776) is widely distributed throughout the Mediterranean and northwestern Atlantic (from the north of Africa to the north of Europe and the Azores). This species has generally been described to be associated with clean gravel or sandy substrata with low algal cover (Holme, 1966; Kanazawa, 1992); however, around the Balearic Islands this species occurs in high abundances in *Peyssonnelia* beds (between 30 m and 100 m depth) (Ordines and Massutí, 2009) where its creates clearly visible furrows (Fig. 1). The present study aimed to elucidate the trophic ecology of *S. purpureus* on sandy bottoms of the Balearic Islands with FA composition analysis, and had three main objectives: a) to elucidate the potential food sources of *S. purpureus* (algae and/or sediment) by comparing the FA profiles; b) to compare the FA profiles of the gonads from locations with different macroalgal communities; and c) to analyze the changes in FA composition with respect to size and gonad biomass, as changes in the diet are thought to affect individual growth and gonad development.

2. Materials and methods

2.1. Study area

The study areas were located at depths between 50 m and 100 m around Mallorca and Menorca (Balearic Islands, western Mediterranean) (Fig. 2). The seabed is composed of soft sediments with or without vegetation. Rhodoliths (corallinaceas) and red algae (*Osmundaria volubilis*, *Phyllophora crispa*, *Peyssonnelia* spp.) dominate this depth range. *S. purpureus* is generally more abundant in coastal soft sediments with red algae (Ordines and Massutí, 2009).

2.2. Sampling method

Samples for the FA composition analysis of gonads were collected over four days (11/13/19/21 May 2009) during the MEDITS0509 survey (Data Collection Framework for the Common Fisheries Policy) on board the R.V. Cornide de Saavedra. A 2-m beam trawl was used to collect the sea urchins and algae. A box-corer was used to obtain sediment samples. Four locations with sandy substrata but with different macroalgal communities were selected (Fig. 2): bare sand (L1) and algal beds on sandy bottoms dominated by rhodoliths and other soft red algae such as *Peyssonnelia* spp., *O. volubilis* and *P. crispa* (L2, L3 and L4). Information on the communities' distribution was based on a previous study (Ordines and Massutí, 2009). At each of the four locations three beam trawl samples were taken 100 m apart and the sea urchin abundances were recorded. General information on the algal and faunal composition was obtained from the same beam trawl samples (Table 1). The sampling design incorporates two factors: location (L1, L2, L3 and L4) and site (each beam trawl sample). A subsample of ten to fifteen individuals was taken at each location and site, and the specimens were measured. The gonads of each individual were removed and weighed. Five gonads of different individuals were randomly selected from each location and site for FA analysis (between approximately 1 and 2 g of the gonad were extracted) (4 locations × 3 sites × 5 replicates). Three subsamples of the three dominant soft algae, *O. volubilis*, *Peyssonnelia* spp. and *P. crispa*, were also obtained from the beam trawl samples at three different sites of vegetated locations (5–6 g) (3 species × 3 sites × 3 replicates). At each location three grabs of sediment were collected, from which two sediment subsamples were taken consisting of 5–6 g of sediment from the first 4 cm of the surface (4 locations × 3 sites × 2 replicates). All samples were frozen in glass tubes with Teflon-lined screw caps, and conserved at −80 °C until FA analysis in the laboratory.

2.3. Laboratory methods

After individual sample/tissue homogenization, the FA composition of the total lipid fraction was determined by fat extraction

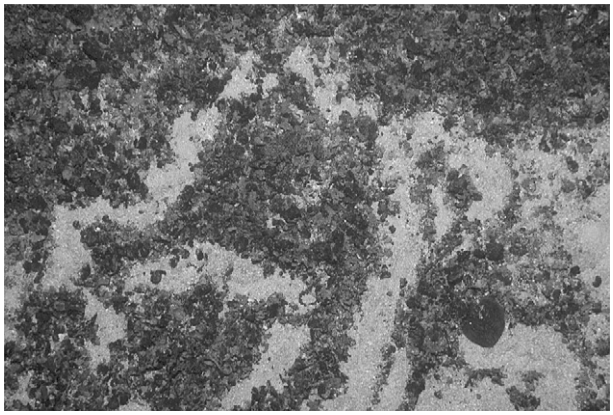

Fig. 1. Photograph taken from a sledge mounted camera showing the tracks made by *Spatangus purpureus* on a vegetated bottom dominated by *Peyssonnelia* spp. and rhodoliths (Corallinacea). Son Bou, SE Menorca, 60 m depth. Photo: F. Sánchez.

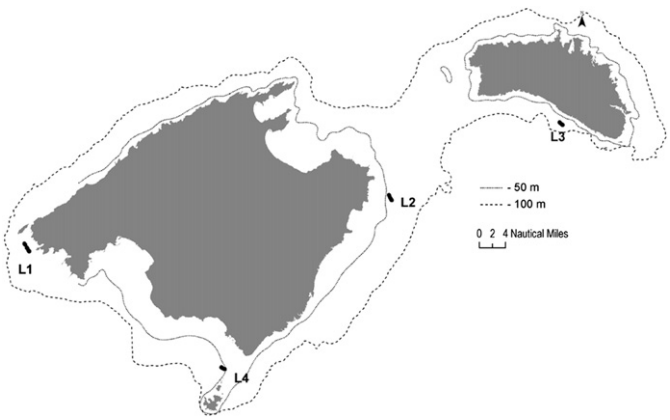

Fig. 2. The selected locations in the study area, circalittoral bottoms around Mallorca and Menorca Islands, between 50 and 100 m.

Table 1
Data on algal and faunal composition (mean ± SE) in the four locations (L1, L2, L3, L4) where the sea urchin were collected. The data were obtained from results of beam trawl samples ($n = 3$).

	Biomass alga (kg/ha)	Corallinaceas (kg/ha)	Other rhodoficea (kg/ha)	Peyssonnelia spp. (kg/ha)	Abundance fauna (ind/ha)	Biomass fauna (kg/ha)	*S. purpureus* (ind/ha)
L1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4467.86 ± 1797.37	49.03 ± 17.90	54.41 ± 32.81
L2	1585.14 ± 316.26	1576.72 ± 316.53	8.40 ± 2.02	0.00 ± 0.00	4042.90 ± 1151.84	134.53 ± 6.49	445.80 ± 148.50
L3	8590.14 ± 1866.50	1344.64 ± 32.61	337.62 ± 32.61	6752.49 ± 652.16	200 428.87 ± 106 193.27	922.40 ± 398.11	696.00 ± 264.00
L4	617.32 ± 42.07	361.65 ± 29.00	255.67 ± 39.05	0.00 ± 0.00	42 262.70 ± 18 956.57	518.57 ± 414.35	2336.00 ± 1877.00

following the method of Folch et al. (1957), with a mixture of chloroform and methanol (1:1 proportion for the first extraction and 2:1 proportion for the second one). Fatty acid methyl ester (FAME) samples were analyzed according to the method of Stoffel et al. (1959) by gas–liquid chromatography using an SP^TM 2560 flexible fused silica capillary column (100-m long, internal diameter of 0.25-mm and film thickness of 0.20 μm; SUPELCO) in a Hewlett–Packard 5890 gas chromatograph. The oven temperature of the gas chromatograph was programmed for 5 min at an initial temperature of 140 °C, and increased at a rate of 4 °C/min to 230 °C, further increased at a rate of 1 °C/min to 240 °C and then held at that temperature for 6 min. The injector and flame ionization detector were set a 250 °C. Helium was used as carrier gas at a pressure of 290 kPa, and peaks were identified by comparing their retention times with appropriate FAME standards purchased from the Sigma Chemical Company (St. Louis, MO, USA). Individual FA concentrations were expressed as percentages of the total content.

2.4. Statistical analysis

Multivariate analysis was carried out using PRIMER v5 (Plymouth Routines In Multivariate Ecological Research) in order to compare the FA profiles of different sample groups (gonads, algae and sediment) and examine the differences within these sample groups. The data were converted into similarity matrices using a *Bray–Curtis* resemblance measure. Permutation-based analysis of similarity (ANOSIM) was used to examine differences in fatty acid profiles across the factors location (for gonads and sediment) and species (for algae). SIMPER analysis (PRIMER 6. software) was used to investigate the similarities and dissimilarities within and between sample groups and the main fatty acids contributing to these (Clarke, 1993). Redundancy Analysis (RDA) was used to test how much of the variance in the multivariate analysis of the FAs in sea urchin gonads (species variables) can be explained by the following factors (environmental variables): location, sea urchin size (S) and gonadosomatic index (GSI = gonad weight/body size). RDA was performed using the software CANOCO (version 4.5) following the procedure established for compositional data (e.g. percentage) using the log-ratio analysis centered both by samples and individual FAs (Aitchison, 1986; Ter Braak and Šmilauer, 2002).

Analysis of variance (ANOVA) was used to test whether the biological parameters of sea urchins (size, gonad weight and GSI by size class), the main FAs and other biochemical parameters (n-3/n-6 ratio, C18:1n-9/C18:1n-7 ratio, ARA/EPA) varied among sea urchin gonads collected at different locations. Biological parameters were analyzed by an unbalanced 1way-ANOVA (factor location with 4 treatments) with $n = 25–50$ individuals. The linear model of FA analysis incorporates two factors: location (fixed, with 4 treatments) and site (random and nested, with 3 treatments). Five replicates were carried out. The FA data are presented as percentages, which require arcsine transformation to produce a normally distributed data set with homogeneous variances (Zar, 1996). Cochran's test was used before the analysis to check for homogeneity of variances in different data populations. When Cochran's test showed significant differences, the significance level of the ANOVA was set at 0.01. When the ANOVA detected significant differences for any factor, the Student Newman–Keuls (SNK) test was applied.

3. Results

3.1. Algal and faunal location characteristics

The four locations selected for this study had sandy bottoms with different algal and faunal communities (Table 1). Location 1 (L1) was typified by bare sandy bottoms without algae. Location 2 (L2) was a vegetated substrate with a high biomass of rhodoliths and other rhodoficies, especially *O. volubilis*. Location 3 (L3) was an algal bed dominated by *Peyssonnelia* spp. and rhodoliths. Location 4 (L4) also had high concentrations of rhodoliths and soft algae, including *P. crispa*, but had a lower algal biomass than L2 and L3. The faunal abundance and biomass were higher in L3 and L4. Sea urchins reached a maximum mean value of 2336.00 ind/ha (=0.2 ind/m^2) in L4. The density of *S. purpureus* was intermediate in L2 and L3 compared with the other locations, and it was very low in L1 (54.41 ind/ha = 0.0054 ind/m^2) (Table 1).

3.2. Biological parameters of S. purpureus

The mean size of *S. purpureus* was 92.20 mm, showing differences between locations and lower mean value in L4, 79.49 mm. Gonad weight also showed significant differences and was higher in L1 and lesser in L4 (Table 2). The size frequency distribution pattern was different according to the location. In L1 sea urchins

Table 2
Value of biological parameters (mean ± SE) of *Spatangus purpureus* collected in four locations (L1, L2, L3, L4) around Mallorca and Menorca Islands. N: number of individuals sampled; GSI: Gonadosomatic Index (size class with a major frequency of individuals). Differences between locations were tested with ANOVA (p = signification level; n.s.: no significant) and S N K test.

Locality	*N*	Size (mm)	Gonad weight (g)	GSI (80–90 cm)	GSI (90–100 cm)
L1	25	99.60 ± 1.89	10.86 ± 0.83	0.06 ± 0.009	0.05 ± 0.004
L2	50	92.66 ± 1.72	6.34 ± 0.76	0.02 ± 0.008	0.03 ± 0.002
L3	41	98.61 ± 1.12	7.40 ± 0.56	0.03 ± 0.002	0.03 ± 0.003
L4	37	79.49 ± 1.60	6.64 ± 0.85	0.05 ± 0.006	0.07 ± 0.003
ANOVA		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
S.N.K.		L1 = L3 > L2 > L4	L1 > L3 = L2 > L4	L1 = L4 > L3 = L2	L4 > L1 = L2 = L3

were always larger than 80 cm, while in L4 there was a high proportion of smaller sea urchins (Fig. 3). The GSI in the different locations was only compared for the size intervals 80–90 cm and 90–100 cm because these were the most frequent in all four habitats. For size class 80–90 cm, the GSI was highest in L1 and L4 and for size class 90–100 cm in L4 (Table 2).

3.3. Fatty acid composition of gonads, sediment and algae

Polyunsaturated fatty acids (PUFAs) dominated the lipids of the sea urchin gonads (41.95 ± 0.77%), while saturated and monounsaturated fatty acids were present in similar percentages (29.73 ± 0.59 and 28.31 ± 0.60% respectively). In algae, saturated FAs dominated the lipid profile (46.28 ± 1.14%) followed by polyunsaturated FAs (37.83 ± 1.37%). Monounsaturated lipids were the least abundant (15.85 ± 0.69%). Sediments had high contents of saturated FAs (47.08 ± 3.62%), while monounsaturated and polyunsaturated FAs were less abundant (34.19 ± 2.27% and 18.72 ± 2.82%, respectively). Of the unsaturated FAs, the *n*-3 moiety predominated in *S. purpureus* gonads (22.93 ± 0.66%), although the *n*-7 and *n*-6 fractions varied in second position with similar proportions (21.03 ± 0.70% and 19.02 ± 0.72% respectively). The *n*-6 FAs (20.68 ± 1.32%) predominated in algae, followed by *n*-3 FAs (17.16 ± 1.61%). Finally, *n*-7 and *n*-9 FAs were also abundant (8.11 ± 0.50% and 7.72 ± 0.45% respectively). The *n*-7 moiety (18.67 ± 2.12%) predominated in sediment, followed, with similar percentages, by *n*-9 (15.50 ± 1.54%), *n*-3 (10.57 ± 2.25%) and *n*-6 FAs (8.14 ± 1.27%) (Table 3).

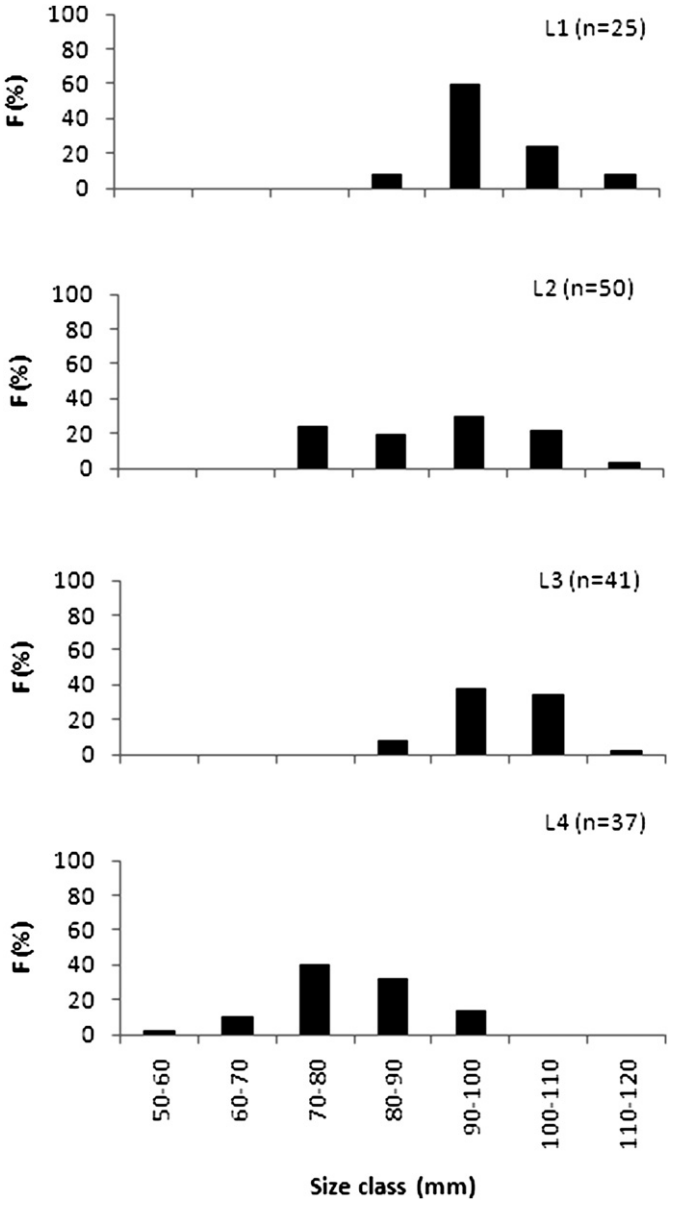

Fig. 3. Size frequency distribution of *Spatangus purpureus* (mm) collected at the four locations (L1, L2, L3, L4) of the circalittoral bottoms around Mallorca and Menorca Islands.

The main FA components in the *S. purpureus* gonads were C20:4*n*-6 (17.46 ± 0.99%), C16:0 (16.32 ± 0.22%), C20:5*n*-3 (15.19 ± 0.63%) and C16:1*n*-7 (13.52 ± 0.66%) (Table 3). However, the ANOSIM test showed that the FA profiles were slightly different between the 4 locations except for L2 and L3 ($R = 0.275$), and was especially significant for L1 and L4 ($R = 0.849$) (Table 4). The main FAs were the most important for the similarity between samples in all cases, but the order of importance of their contribution changed with the location (Table 5). The dissimilarity was mainly due to the proportions of C16:1*n*-7, C20:4*n*-6 and C20:5*n*-3, and secondarily due to C22:6*n*-3 and C14:0, which marked the differences between L1 and the rest of the locations (SIMPER, Table 4). The main FAs in algae were C16:0 (33.70 ± 1.52%), C20:4*n*-6 (17.50 ± 0.86%) and C20:5*n*-3 (9.00 ± 0.86%) (Table 3); however, the FA profile changed between species ($R = 0.691$), and the differences between *O. volubilis* and *Peyssonnelia* sp. ($R = 0.952$) were the most significant. The dissimilarity between samples was explained by these FAs and C22:6*n*-3. This FA was more important in *O. volubilis* and *P. crispa* than in *Peyssonnelia* sp., while in this algae C16:0 was higher (Table 3). Moreover, C16:0 was the main component in the FA composition of sediment (26.74 ± 0.69%), followed by C18:1*n*-9 (14.40 ± 1.12%) and C16:1*n*-7 (13.42 ± 1.01%) (Table 3). In this case, the differences between locations were not significant due to the high variation between replicates ($R = 0.174$, Table 4), except for L1 and L4 ($R = 6.626$; Table 4). The dissimilarity in this case was due to variation in C18:1*n*-9, C16:1*n*-7 and C20:4*n*-6. The two first FAs were highest in L1, while the third in L4 (Table 3).

3.4. Changes in fatty acid composition in gonads in relation to location and biological parameters

The RDA results showed that two explanatory variables in the model were significant: location and gonadosomatic index (GSI). Location explained 64.3% of the variance ($p = 0.002$) (Fig. 4, Table 6). The largest proportion of the variance on the correlation with the first PCA components was explained by the algae biomass of the sampled locations, in a gradient from locations with less algae biomass (L1 and L4) to locations with more algal biomass (L2 and L3). However, this variance was also explained by the different FA profile of L4 samples. Particular FAs contributes to variance between locations (Fig. 4). The univariate analysis (ANOVA) applied to FAs helped to define the changes in the FA proportions in relation to location (Fig. 5). The proportion of FAs C14:0 and C22:6*n*-3 was significantly higher in the gonads extracted from sea urchins collected at L1 (sand). C16:1*n*-7, C18:1*n*-9 and C20:5*n*-3 were more abundant in the gonads of sea urchins from L4, a location with a lower algal biomass, while C20:4*n*-6 was less abundant. There was a lower proportion of C18:1*n*-7 at L2 (rhodoliths and soft algae, such as *O. volubilis*). The FAs C15:0, C17:0, C20:0, C17: 1*n*-7, C18:2*n*-6, C22:2*n*-6 and C24:1*n*-9 were also relevant for the variation in the FA profile in relation to location (Fig. 4), but they represented only a small proportion of the FA composition (<5%) (Table 3). The 2nd explanatory variable, GSI, accounted for only 7.1% ($p = 0.002$) of the explained variance of the model in the RDA analysis (Table 6, Fig. 4).

Table 3
Fatty acid composition (mean ± standard error) of total lipids extracted from gonads of *Spatangus purpureus* collected in different locations (L1, L2, L3 and L4). Also the FA composition of the main algae species (Os = *Osmundaria volubilis*, Pe = *Peyssonnelia* sp., Ph = *Phyllophora* crispa) and the sediment collected in each location is shown.

	Gonads of *Spatangus purpureus*				Algae			Sediment			
	L1 ($n = 15$)	L2 ($n = 15$)	L3 ($n = 15$)	L4 ($n = 15$)	Os ($n = 9$)	Pe ($n = 9$)	Ph ($n = 9$)	L1 ($n = 6$)	L2 ($n = 6$)	L3 ($n = 6$)	L4 ($n = 6$)
C12:0	0.08 ± 0.02	0.25 ± 0.20	0.06 ± 0.01	0.08 ± 0.01	0.56 ± 0.11	0.64 ± 0.16	0.54 ± 0.19	0.11 ± 0.03	0.05 ± 0.03	0.03 ± 0.02	0.00 ± 0.00
C13:0	0.10 ± 0.03	0.01 ± 0.01	0.15 ± 0.02	0.09 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.03	0.05 ± 0.03	0.19 ± 0.12	0.00 ± 0.00
C14:0	8.87 ± 0.16	5.51 ± 0.20	5.67 ± 0.26	5.43 ± 0.44	4.42 ± 0.21	3.31 ± 0.09	4.29 ± 0.21	5.18 ± 0.16	3.84 ± 0.59	3.32 ± 0.47	2.38 ± 0.36
C14:1*n*-5	0.34 ± 0.02	0.38 ± 0.05	0.63 ± 0.15	0.45 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C15:0	2.55 ± 0.06	1.29 ± 0.08	1.72 ± 0.21	0.83 ± 0.17	0.41 ± 0.06	0.40 ± 0.11	0.97 ± 0.09	2.80 ± 2.65	1.15 ± 0.45	1.51 ± 0.32	1.56 ± 0.27
C15:1*n*-5	0.02 ± 0.01	0.10 ± 0.08	0.21 ± 0.06	0.13 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
C16:0	14.50 ± 0.23	17.14 ± 0.25	16.70 ± 0.32	15.91 ± 0.43	26.70 ± 0.34	43.19 ± 1.36	31.21 ± 0.80	26.42 ± 0.70	26.49 ± 1.20	22.65 ± 2.34	27.74 ± 0.88
C16:1*n*-7	11.33 ± 0.16	15.34 ± 0.92	12.65 ± 1.04	18.89 ± 0.46	4.79 ± 0.23	5.09 ± 0.30	3.58 ± 0.17	10.72 ± 0.89	14.62 ± 0.93	10.55 ± 1.67	16.00 ± 1.44
C17:0	1.32 ± 0.11	1.57 ± 0.39	1.19 ± 0.16	0.59 ± 0.05	0.44 ± 0.07	0.18 ± 0.05	0.70 ± 0.03	1.25 ± 0.05	1.11 ± 0.11	1.10 ± 0.11	0.86 ± 0.13
C17:1*n*-7	1.00 ± 0.03	1.45 ± 0.17	0.94 ± 0.10	0.35 ± 0.11	0.33 ± 0.12	0.36 ± 0.18	0.30 ± 0.16	0.63 ± 0.26	0.57 ± 0.15	2.00 ± 0.75	0.96 ± 0.18
C18:0	3.50 ± 0.09	4.01 ± 0.33	3.42 ± 0.23	2.82 ± 0.14	6.44 ± 0.27	5.30 ± 0.17	6.48 ± 0.31	9.03 ± 0.33	7.66 ± 0.45	22.31 ± 7.82	8.04 ± 0.34
C18:1*n*-5	1.83 ± 0.20	2.67 ± 0.16	3.16 ± 0.26	1.90 ± 0.18	0.11 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C18:1*n*-7	6.48 ± 0.10	4.12 ± 0.22	5.89 ± 0.23	5.67 ± 0.28	3.58 ± 0.06	3.31 ± 0.33	3.00 ± 0.26	2.78 ± 0.22	5.10 ± 0.89	4.75 ± 0.58	6.28 ± 0.38
C18:1*n*-9	1.84 ± 0.15	1.64 ± 0.12	1.71 ± 0.13	3.79 ± 0.36	5.02 ± 0.19	8.47 ± 0.37	7.51 ± 0.19	19.97 ± 0.82	11.78 ± 1.28	12.77 ± 1.58	10.68 ± 0.96
C18:2*n*-6	0.65 ± 0.07	1.79 ± 0.21	1.68 ± 0.26	0.77 ± 0.09	2.14 ± 0.22	2.79 ± 0.20	1.50 ± 0.08	4.63 ± 0.47	3.11 ± 0.23	3.49 ± 0.47	3.71 ± 0.33
C18:3*n*-3	0.70 ± 0.05	0.53 ± 0.08	0.96 ± 0.15	0.90 ± 0.05	0.09 ± 0.05	0.04 ± 0.03	0.39 ± 0.18	0.00 ± 0.00	0.13 ± 0.08	1.31 ± 0.42	1.33 ± 0.30
C18:3*n*-6	0.10 ± 0.03	0.38 ± 0.07	0.24 ± 0.04	0.30 ± 0.04	0.06 ± 0.03	0.00 ± 0.00	0.03 ± 0.02	0.00 ± 0.00	0.23 ± 0.07	0.00 ± 0.00	0.36 ± 0.10
C18:4*n*-3	0.03 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.67 ± 0.10	0.04 ± 0.03	0.51 ± 0.11	0.06 ± 0.04	0.33 ± 0.19	0.00 ± 0.00	0.47 ± 0.10
C20:0	0.77 ± 0.06	0.42 ± 0.05	0.40 ± 0.07	0.25 ± 0.06	0.59 ± 0.13	0.17 ± 0.08	0.38 ± 0.08	1.27 ± 0.10	0.90 ± 0.06	1.04 ± 0.05	0.98 ± 0.13
C20:1*n*-9	0.73 ± 0.05	0.64 ± 0.09	1.08 ± 0.14	0.82 ± 0.06	0.47 ± 0.12	0.42 ± 0.30	0.78 ± 0.09	0.10 ± 0.06	0.41 ± 0.14	0.00 ± 0.00	0.34 ± 0.12
C20:2*n*-6	1.40 ± 0.03	1.34 ± 0.14	1.08 ± 0.18	0.98 ± 0.05	0.54 ± 0.08	0.31 ± 0.12	0.30 ± 0.10	0.00 ± 0.00	0.48 ± 0.12	0.14 ± 0.09	0.00 ± 0.00
C20:3*n*-3	0.09 ± 0.03	0.03 ± 0.02	0.34 ± 0.06	0.20 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
C20:3*n*-6	0.41 ± 0.02	0.54 ± 0.09	0.49 ± 0.03	0.35 ± 0.07	0.92 ± 0.26	0.18 ± 0.07	0.18 ± 0.07	0.00 ± 0.00	0.19 ± 0.11	0.00 ± 0.00	0.10 ± 0.06
C20:4*n*-6 (ARA)	15.68 ± 0.37	17.07 ± 1.03	19.67 ± 0.87	9.76 ± 0.29	17.67 ± 0.65	15.24 ± 1.07	19.58 ± 1.43	1.22 ± 0.30	4.89 ± 1.04	3.31 ± 0.47	5.19 ± 0.36
C20:5*n*-3 (EPA)	15.11 ± 0.61	14.13 ± 0.74	11.94 ± 0.46	22.12 ± 1.08	14.44 ± 0.80	6.36 ± 0.36	6.20 ± 0.41	6.29 ± 0.45	9.31 ± 1.92	5.29 ± 0.62	8.45 ± 0.42
C21:0	0.20 ± 0.09	0.24 ± 0.09	0.26 ± 0.10	0.04 ± 0.04	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
C22:0	0.11 ± 0.04	0.15 ± 0.05	0.35 ± 0.05	0.17 ± 0.02	0.37 ± 0.06	0.44 ± 0.16	0.71 ± 0.06	1.59 ± 0.15	1.29 ± 0.14	1.30 ± 0.15	1.46 ± 0.22
C22:1*n*-9	0.47 ± 0.02	0.38 ± 0.07	0.56 ± 0.10	0.23 ± 0.06	0.00 ± 0.00	0.04 ± 0.03	0.00 ± 0.00	1.02 ± 0.24	2.32 ± 0.48	1.06 ± 0.16	0.87 ± 0.25
C22:2*n*-6	0.01 ± 0.01	0.00 ± 0.00	0.17 ± 0.03	0.41 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.05	0.31 ± 0.09	0.37 ± 0.13	0.24 ± 0.10	0.00 ± 0.00
C22:4*n*-6	0.10 ± 0.07	0.21 ± 0.12	0.25 ± 0.08	0.27 ± 0.02	0.22 ± 0.09	0.32 ± 0.14	0.00 ± 0.00	0.37 ± 0.12	0.26 ± 0.09	0.22 ± 0.09	0.00 ± 0.00
C22:5*n*-3	1.03 ± 0.03	1.66 ± 0.21	0.80 ± 0.11	0.59 ± 0.05	0.58 ± 0.12	0.06 ± 0.05	0.24 ± 0.13	0.99 ± 0.25	0.71 ± 0.19	0.00 ± 0.00	0.00 ± 0.00
C22:6*n*-3 (DHA)	7.65 ± 0.07	3.61 ± 0.15	4.66 ± 0.21	4.63 ± 0.23	8.24 ± 0.88	3.28 ± 0.29	10.34 ± 2.14	2.46 ± 0.53	2.06 ± 0.52	1.16 ± 0.56	2.24 ± 0.83
C23:0	0.02 ± 0.01	0.00 ± 0.00	0.14 ± 0.03	0.03 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.37 ± 0.12	0.23 ± 0.08	0.28 ± 0.10	0.00 ± 0.00
C24:0	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C24:1*n*-9	1.01 ± 0.05	1.37 ± 0.28	0.82 ± 0.10	0.23 ± 0.04	0.16 ± 0.09	0.04 ± 0.03	0.24 ± 0.09	0.38 ± 0.16	0.19 ± 0.07	0.00 ± 0.00	0.00 ± 0.00
n-3	24.60 ± 0.60	19.95 ± 0.64	18.69 ± 0.47	28.45 ± 0.94	24.01 ± 0.66	9.80 ± 0.54	17.68 ± 2.55	6.92 ± 0.91	12.62 ± 2.41	7.76 ± 1.30	12.48 ± 1.03
n-5	0.36 ± 0.03	0.47 ± 0.09	0.84 ± 0.20	0.57 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
n-6	18.33 ± 0.36	21.33 ± 1.20	23.59 ± 0.98	12.84 ± 0.35	21.55 ± 0.58	18.84 ± 1.08	21.65 ± 1.40	8.04 ± 0.58	9.53 ± 0.88	7.39 ± 0.88	9.36 ± 0.48
n-7	18.82 ± 0.18	20.91 ± 1.12	19.49 ± 0.98	24.91 ± 0.47	8.70 ± 0.35	8.76 ± 0.50	6.89 ± 0.30	2.56 ± 0.45	20.29 ± 0.73	17.30 ± 2.29	23.24 ± 1.35
n-9	4.04 ± 0.13	4.04 ± 0.37	4.18 ± 0.20	5.07 ± 0.32	5.65 ± 0.23	8.98 ± 0.66	8.52 ± 0.15	3.11 ± 0.67	14.71 ± 1.02	13.82 ± 1.67	11.89 ± 1.18
Saturates	32.01 ± 0.41	30.63 ± 0.56	30.05 ± 0.44	26.24 ± 0.99	39.97 ± 0.21	53.63 ± 1.15	45.27 ± 1.30	48.07 ± 1.83	42.78 ± 3.05	53.72 ± 8.57	43.03 ± 2.05
Monounsaturates	25.05 ± 0.22	28.09 ± 0.74	27.66 ± 0.84	32.45 ± 0.60	14.46 ± 0.36	17.73 ± 0.88	15.41 ± 0.37	35.55 ± 1.67	35.06 ± 1.84	31.12 ± 5.86	35.14 ± 0.40
Polyunsaturates	42.94 ± 0.48	41.28 ± 0.83	42.29 ± 0.97	41.29 ± 0.82	45.56 ± 0.45	28.64 ± 1.29	39.32 ± 1.44	16.32 ± 2.18	22.15 ± 4.40	15.16 ± 3.31	21.84 ± 2.15
n-3/*n*-6	1.36 ± 0.05	1.00 ± 0.09	0.81 ± 0.04	2.25 ± 0.11	1.14 ± 0.06	0.54 ± 0.04	1.06 ± 0.29	0.46 ± 0.13	1.44 ± 0.34	1.19 ± 0.15	1.33 ± 0.08
EPA/DHA	1.98 ± 0.09	4.02 ± 0.27	2.62 ± 0.13	5.05 ± 0.43	2.13 ± 0.26	2.16 ± 0.23	0.81 ± 0.09	0.38 ± 3.75	2.15 ± 0.54	0.79 ± 0.45	1.49 ± 0.54
C18:1*n*-9/C18:1*n*-7	0.28 ± 0.01	0.44 ± 0.08	0.29 ± 0.06	0.67 ± 0.02	1.40 ± 0.02	2.56 ± 0.10	2.50 ± 0.17	7.17 ± 0.45	2.31 ± 0.26	2.69 ± 0.74	1.70 ± 0.32

Table 4
ANOSIM of fatty acids profile for all the samples (All), between the different locations where sea urchins and sediment samples were collected (L1, L2, L3, L4) and between different species of algae (Os = *Osmundaria volubilis*; Pe = *Peyssonnelia* sp.; Ph = *Phyllophora crispa*).

	Gonads		Sediment			Algae	
	R	*p*	*R*	*p*		*R*	*p*
All	0.562	0.001	0.174	<0.01	All	0.691	0.001
L1 & L2	0.62	0.001	0.243	<0.05	Os & Pe	0.952	0.001
L1 & L3	0.572	0.001	0.122	<0.01	Os & Ph	0.544	0.001
L1 & L4	0.849	0.001	0.626	<0.01	Pey & Ph	0.565	0.001
L2 & L3	0.275	0.001	−0.043	n.s			
L2 & L4	0.672	0.001	0.004	n.s.			
L3 &L 4	0.554	0.001	0.08	n.s.			

In the RDA graph GSI variable appears represented between locations with less algal biomass (L1 and L4), locations where sea urchins showed the more high gonadosomatic index. The most representative FA for explaining this variance was C18:1*n*-7, which also showed significant differences in relation to location, and was lower at L2 (Fig. 5).

In general, saturated and polyunsaturated FAs were similar at all the locations (Table 3), and only the proportion of monounsaturated FAs was slightly higher at L4 (sand with lower algal biomass) (ANOVA, $p < 0.001$, S.N.K.: L4 > L3 = L2 > L1) (Table 3). The *n*-3 moiety was significantly higher in the gonads of *S. purpureus* collected at L4 compared with the other locations, followed by L1 (bare sand). There was a higher proportion of the *n*-7 moiety in the gonads from L4. However, the *n*-6 moiety was less abundant in this location; therefore, the ratio *n*-3/*n*-6 also showed significant differences in relation to location (Fig. 5). The ratio C18:1 (*n*-9/*n*-7) was higher at L4 (bare sand) and L2 (rhodoliths and soft algae such as *O. volubilis*) and the proportion of EPA/DHA was lower at L4 (Fig. 5).

4. Discussion

The current study shows that the FA profile of *S. purpureus* gonads can be a useful trophic marker, as it was in good agreement with the FA composition of the potential trophic resources in the benthos from which the sea urchins obtain their food. The FA signature varied between locations, and reflected the availability of food resources and possible dietary adaptations (Ginger et al., 2000; Sargent et al., 1999, 2002). Another factor affecting the FA profile is gonadal development (Cook et al., 2007). The results evidences that *S. purpureus* feeds on a wide range of potential food (phytodetritus, faunal detritus and bacterial mats), which is clearly indicative of an omnivorous diet.

4.1. FA composition of S. purpureus and potential food sources

The FA profiles of *S. purpureus* gonads show large proportions of palmitic (C16:00), arachidonic (C20:4*n*-6), eicosapentaenoic (C20:5*n*-3) and vaccenic (C16:1*n*-7) acids. The pattern was similar in algae, except for vaccenic acid, which was common in the sediment samples. The FA composition in *S. purpureus* can be interpreted as a combination of fatty acids of vegetal origin (algal detritus), animal origin (infaunal and faunal detritus) and others related to sediment (organic matter, bacteria). In general, PUFAs explain a vegetal or animal origin in the diet, being predominant components in the lipids of higher plants and animals. However, another possible origin is possible and is discussed in the next sections. Saturated and monounsaturated FA dominated in sediment samples, explained for the FA composition of bacteria, where PUFA are absent (Lenninger, 1984). Saturated and monounsaturated

Table 5
Results of SIMPER analysis showing average similarity within the samples groups (S) and average dissimilarity between samples groups (D). The analysis was carried out for all the samples (All), for locations were sea urchins and sediment were collected (L1, L2, L3, L4) and for the main algae species (Os = *Osmundaria volubilis*, Pe = *Peyssonnelia* sp., Ph = *Phyllophora crispa*). The contribution of the fatty acids to the respective groups is shown as a percentage of the total (%).

Similarity						Dissimilarity				
Gonads	All $S = 85.49\%$	L1 $S = 93.71\%$	L2 $S = 86.08\%$	L3 $S = 87.54\%$	L4 $S = 89.00\%$	Gonads	L1 & L2 $S = 17.80\%$	L1 & L3 $S = 15.57\%$	L1 & L4 $S = 21.07\%$	L2 & L4 $S = 20.31\%$	L3 & L4 $S = 22.45\%$
C20:4*n*-6	18.02	15.89	17.15	20.44	10.27	C16:1*n*-7	13.01	11.2	17.96	9.89	13.94
C16:0	17.83	14.96	19.3	18.33	16.72	C22:6*n*-3	11.39	9.64	7.17		
C20:5*n*-3	14.37	14.71	14.59	12.53	22.03	C20:4*n*-6	10.27	13.29	14.05	18.13	22.1
C16:1*n*-7	13.09	11.74	15.51	12.1	20.08	C14:0	9.47	10.32	8.18		
						C20:5*n*-3	8.74	11.64	16.9	20.34	22.73
Algae	All $S = 80.60\%$	Os $S = 87.63\%$	Pe $S = 86.86\%$	Ph $S = 84.52\%$		Algae	Os & Pe $S = 24.95\%$	Os & Ph $S = 19.00\%$	Pe & Ph $S = 21.25\%$		
C16:0	36.30	29.54	46.1	34.85		C16:0	33.05	12.51	28.2		
C20:4*n*-6	18.51	18.37	14.73	19.73		C20:5*n*-3	16.19	21.04			
C20:5*n*-3	8.14	14.27	6.39	6.26		C22:6*n*-3	9.92	15.56	16.7		
						C20:4*n*-6	8.68	13.23	15.6		
Sediment	All $S = 73.91\%$	L1 $S = 84.53\%$	L2 $S = 75.26\%$	L3 $S = 62.22\%$	L4 $S = 81.42\%$	Sediment	L1 & L4 $S = 24.21\%$				
C16:0	31.31	29.31	32.27	28.87	31.56	C18:1*n*-9	19.17				
C18:1*n*-9	14.88	21.41	12.98	14.67	10.48	C16:1*n*-7	13.64				
C16:1*n*-7	13.27	10.2	17.16	10.54	15.44	C20:4*n*-6	8.21				

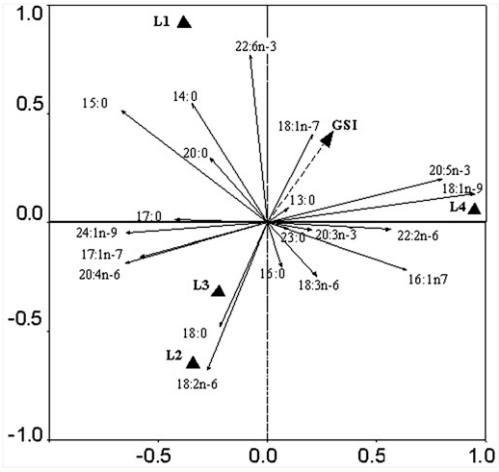

Fig. 4. Redundancy analysis (RDA) biplots of the fatty acid composition (%) in *Spatangus purpureus* gonads. Only the fatty acids that contributed most to the dissimilarity between groups are included. L1, L2, L3, L4 are the different locations where the sea urchins were collected.

have also been defined as major components in algae (Ackman, 1981), but in the current work also arachidonic and eicosapentaenoic have been defined as the main FA in algae, consistent with others studies on red algae (Khotimchenko et al., 2002; Nelson et al., 2002). Diverse PUFA have been identified as qualitative markers of fatty acids of vegetal origin in the trophic interactions in benthic and pelagic ecosystems (Table 7).

Even though palmitic acid (C16:0) is an important component of the lipid fraction in the gonads of *S. purpureus* and especially abundant in algae and sediment, it cannot be considered an interesting trophic marker because it is present in high proportions in many organisms (Řezanka and Sigler, 2009). However, this FA is less abundant in the gonads of *S. purpureus* collected on bare sand bottoms, which could be related to low algal availability. Palmitic acid can generate estearic acid (C18:0), which is used as a precursor of monounsaturated FA by desaturation processes (formation of double bonds). In animal tissues the most common monounsaturated FAs are palmitoleic acid (C16:1*n*-7) and oleic acid (C18:1*n*-9), both precursors in the formation of polyunsaturated FAs. Only the former FA was found in large proportions in both the sea urchin and sediment samples, which suggests that it can be used as a trophic marker for mud ingesters (Cook et al., 2000). Other FAs are also necessary for the formation of PUFAs, such as linoleic acid (C18:2n-6) and alpha-linolenic acid (C18:3*n*-3), which belong to the group of essential FAs (Lenningher, 1984). These FAs make up only a small part of the composition of *S. purpureus* but they were used as diet markers for mud ingesters and herbivorous organisms respectively (Table 7). However, this species may be able to transform these essential FAs into the polyunsaturated FAs C20 and C22, which has been demonstrated in other sea urchin species (Bell et al., 2001).

Table 6
Results of the redundancy analysis for the relative contribution of the fatty acids found in the gonads of the *Spatangus purpureus*. The full model contains all the variables included in the model: Location, Size and the Gonadosomatic Index (GSI). The explained variance (EV) for the full model and each variable after extracting the effect of the covariables is also indicated.

Effect	Covariance	Trace	EV	*F*-ratio	*P*-value
Full model		0.42	42.0%	7.82	0.001
Location	Size, GSI	0.27	64.3%	8.22	0.002
Size	Location, GSI	0.01	2.4%	0.72	0.747
GSI	Location, Size	0.03	7.1%	7.14	0.010

4.2. Origin of vegetal fatty acids

In general, PUFAs can be considered as trophic markers of a photosynthetic origin in the diet (Cook et al., 2000; Brett and Müller-Navarra, 1997; Ikawa, 2004) and are predominant components in the lipids of higher plants and animals. Eicosapentaenoic acid (C20:5*n*-3) and arachidonic acid (C20:4*n*-6) were found in large proportions in the gonads of sea urchins and the algae analyzed, and are representative components in the FA profile of herbivorous organisms (Table 7). They are also essential FAs and serve as precursors to eicosanoids, which are critical in a large range of physiological processes (Lenningher, 1984). In general, they are present in considerable amounts in sea urchins that feed on algae (Isay and Busarova, 1984; Cook et al., 2000; Hughes et al., 2005) and some macroalgal species (Paradis and Ackman, 1977; Khotimchenko et al., 2002; Nelson et al., 2002). Eicosapentaenoic acid (C20:5*n*-3) is characteristic of marine invertebrates (Giddings and Hill, 1975) and is especially abundant in echinoderms (holothurians) (Isay and Busarova, 1984; Romashina, 1983). In *S. purpureus* gonads it is the third most important FA (11–22%), and there is a higher proportion in the gonads of sea urchins from locations dominated by soft red algae (L4). Arachidonic acid, ARA (C20:4*n*-6), is well represented in the gonads of *S. purpureus* from all the locations, although its proportion is lower in the sea urchins collected at the locations with a low algal biomass. Cook et al. (2000) found a predominance of this FA and stearidonic acid (18:4*n*-3) in the sea urchin *Psammechinus miliaris*, whose diet is mainly composed of the alga *Laminaria saccharina*. High levels of EPA and ARA have also been found in other macroalgal species (e.g. *Laminaria digitata*, *Alaria esculenta*) (Mai et al., 1996), and it has been suggested that they are indicative of macroalgal material in the diet of marine organisms (Sargent et al., 1987). Isay and Busarova (1984) found that high levels of arachidonic acid are generally found in urchins and starfish, which could indicate that these organisms have the ability to accumulate it in large quantities (Takagi et al., 1980). However, stearidonic acid is not a representative FA in any of the elements analyzed in the current work, suggesting that probably it is produced by the urchins themselves.

Some long-chain FAs are synthesized from the short chain precursor FAs, and therefore they are not necessarily present in the diet (Dalsgaard et al., 2003; Sargent et al., 1987). Linoleic acid and linolenic acid are only produced by vegetal organisms, and EPA and DHA are either obtained directly from a vegetal origin or by converting from linolenic acid (Brett and Müller-Navarra, 1997). For example, *Psammechinus miliaris* is capable of producing the PUFAs C20 and C22 from the short chain precursor linoleic acid (C18:2*n*-6), although the formation rate of eicosapentaenoic (C20:5*n*-3) in *P. miliaris* is slow, equivalent to only 0.009% of linoleic ingested over a 14-d period. Although sea urchins and many organisms can convert linoleic and alpha-linolenic acid (C18:3*n*-3) to EPA and DHA, this conversion seems to be inefficient for maintaining optimal growth rates. The changes in the proportion of these HUFA (high unsaturated fatty acids) in *S. purpureus* in relation to location suggest that the main origin is accumulation from the diet. The possibility of biosynthesis of these PUFAs by either free-living or endosymbiotic bacteria has been investigated in hydrothermal worms and fish intestines (Pond et al., 2002; Yano et al., 1997). There are still no studies on the microbial biochemistry of *S. purpureus*, but it probably plays an important role in the nutrition and metabolism of the sea urchin as has been found for other spatangoids (Buchanan et al., 1980; Brigmon and Ridder, 1998; Temara et al., 1991, 1993).

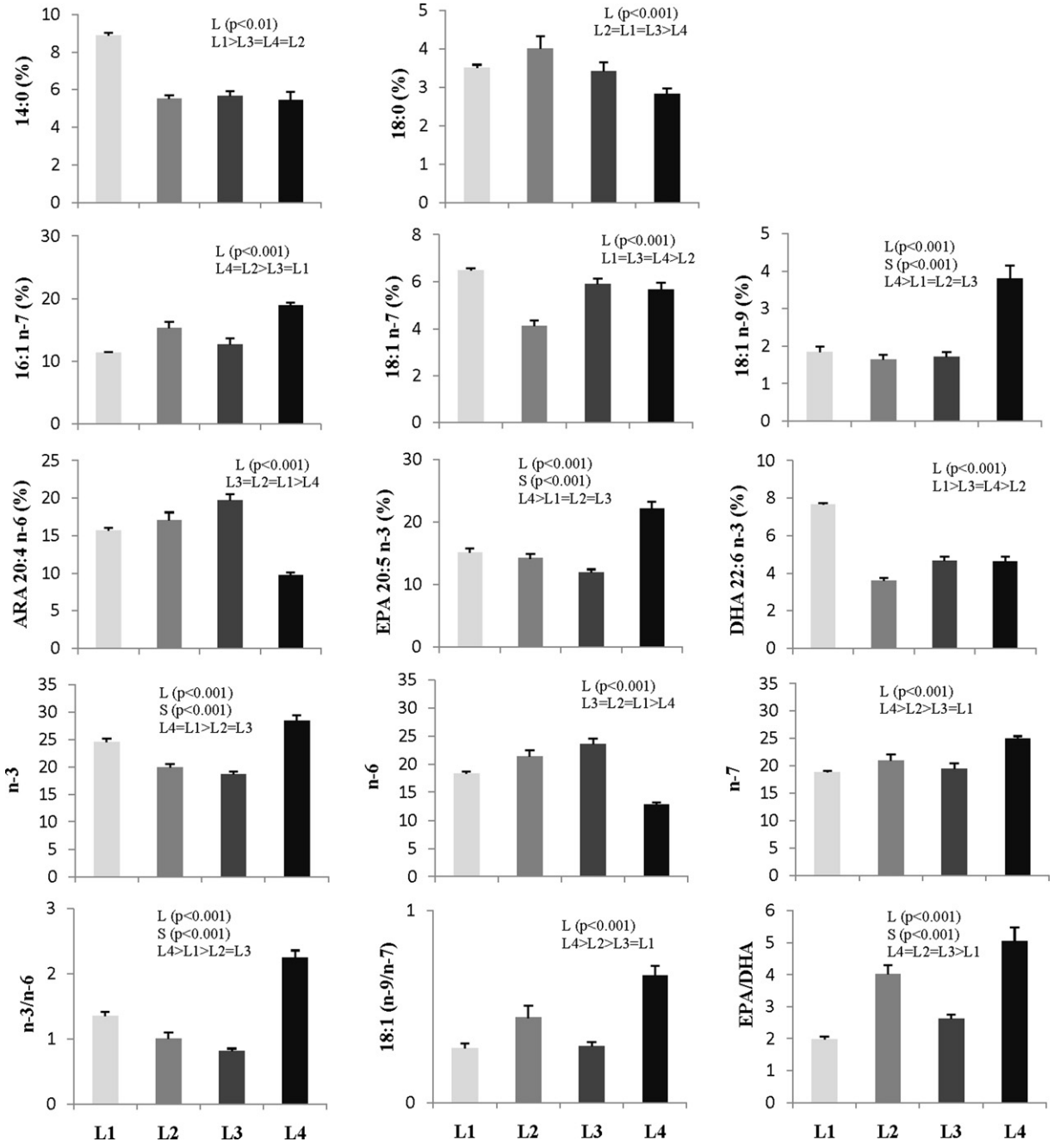

Fig. 5. Values (mean ± SE) of the main fatty acids in the total lipids extracted from the gonads of *Spatangus purpureus*, showing significant differences between locations (L1, L2, L3, L4).

4.3. Trophic markers of bacterial input and carnivorous diet

A high proportion of palmitoleic acid (16:1*n*-7) and vaccenic acid (18:1*n*-7) and a low oleic/vaccenic (C18:1*n*-9/n-7) ratio have been used to indicate the importance of the bacterial input to the diet (Parkes, 1987; Sargent et al., 1987; Kharlamenko et al., 1995; Pond et al., 2002; Cook et al., 2000). In this work palmitoleic acid is also characteristic of sediment samples, and relatively abundant in all sea urchins. In addition, the oleic/vaccenic ratio is always inferior to 1% and significantly different between the sea urchin gonad samples collected at different locations. The lowest ratio values were found for bare sand bottoms. This result provides evidence that, although the algal food source is important, bacterial inputs are also necessary in order to account for the FA signature found in *S. purpureus*.

A large proportion of docosahexaenoic acid (C22:6*n*-3) is related to a carnivorous/necrophagus diet in benthic organisms (Pond et al., 2002; Cook et al., 2000, 2007; Gunstone et al., 1994). For example, myristic acid (C14:0) and docosahexaenoic acid are significantly more abundant in the gonads of sea urchins collected on sand. In this location, where there is little phytodetritus, sea urchins exploit the food that is available and obtain the PUFA necessary for their metabolism by ingesting infauna or animal detritus. Moreover, an increase in protein content in the diet can promote the accumulation of docosahexaenoic acid and arachidonic acid as the gonadal tissue matures (Cook et al., 2007). In fact,

Table 7
Revision on the information on the main fatty acids used as trophic marker in benthos ecology.

Fatty acid	Common name	Trophic marker for	References
C22:6*n*-3	Docosahexaenoic (DHA)	Dinoflagelates; Suspension or filter feeding	Howell et al., 2003; Sargent et al., 1987; Sargent and Henderson, 1995. Vashappilly and Chen, 1998; Mansour et al., 1999; Hughes et al., 2005
		Zooplankton	Kharlamenko et al., 2001
		Carnivorous feeding	Takagi et al., 1980; Kharlamenko et al., 2001; Cook et al., 2000; Budge et al., 2002; Hughes et al., 2005
C20:5*n*-3	Eicosapentaenoic (EPA)	Diatomeas; Suspension feeding	Howell et al., 2003
		Macroalgal and sea urchins feeding on macroalgal	Ackman et al., 1968, Paradis and Ackman, 1977; Isay and Busarova, 1984; Kayama et al., 1989; Mai et al., 1996; Cook et al., 2000; Nelson et al., 2002; Hughes et al., 2005
C20:4*n*-6	Arachidonic (ARA)	Protozoans, Microeucariotes in the sediment	Howell et al., 2003
		Macroalgal and sea urchins feeding on macroalgal	Paradis and Ackman, 1977; Cook et al., 2000; Isay and Busarova, 1984; Mai et al., 1996; Nelson et al., 2002; Hughes et al., 2005
C18:2*n*-6	Linoleic	Mud ingesters	Howell et al., 2003
		Seagrass	Kharlamenko et al., 2001
		Algae	Nichols et al., 1982; Kayama et al., 1989; Nelson et al., 2002;
C18:1*n*-9	Oleic	Carnivorous feeding	Howell et al., 2003
C18:1*n*-7	Vaccenic	Bacteria; Mud ingester	Volkman et al., 1980; Pond et al., 2002; Howell et al., 2003
C18:3*n*-3	Alpha-linolenic	Herbivorous diet	Sargent et al., 1987; Mai et al., 1996; Nelson et al., 2002
		Seagrass	Kharlamenko et al., 2001
		Algae	Nichols et al., 1982; Kayama et al., 1989; Nelson et al., 2002
C18:4*n*-3	Stearidonic	Herbivorous	Sargent et al., 1987; Mai et al., 1996
		Sea urchins feeding on algae	Cook et al., 2000; Hughes et al., 2005
		Algae	Kayama et al., 1989
C16:1*n*-7	Palmitoleic	Bacteria; Mud ingesters;	Cook et al., 2000; Gillian et al., 1988
		Diatoms	Ackman et al., 1968
		Algae	Nelson et al., 2002
C18:1 (*n*-9/*n*-7)	Ratio Oleic/Vaccenic	Measure of bacterial input in the diet	Howell et al., 2003; Cook et al., 2000

the GSI was higher in sea urchins collected on sandy bottoms without algae or with low biomass. The sea urchins collected in the present study were sampled at the same time of year and at similar depths to minimize any temporal and bathymetric effects on the FA profiles (Budge et al., 2002; Lewis, 1967; Fergurson, 1976; Hughes et al., 2005). Therefore, differences in the gonadal index and sizes could be explained by differences in growth or reproductive development due to food availability and geographical differences (Harrold and Reed, 1985; Budge et al., 2002).

Current evidence suggests that not only DHA but also a balanced proportion of the eicosapentaenoic/docasahexaenoic (EPA/DHA) acids in the diet can promote fish growth (Sargent et al., 1999; Izquierdo et al., 2001). Several studies have recognized the importance of considering a balanced ratio of these essential fatty acids in the nutrition of aquaculture species, and larger sizes were obtained in the culture of fish larvae when the ratio value was reduced by incorporating DHA (Verreth et al., 1994; Izquierdo, 1996; Izquierdo et al., 2001). The results of this work support this hypothesis because the value of this ratio was lower in sea urchins collected on bare sand bottoms (L1) due to higher DHA values. Sea urchins were also bigger in this location in comparison with the other locations with algal beds (L2 and L4). Sea urchins are unable to synthesize DHA *de novo* (Bell et al., 1986; Sargent et al., 2002; Castell et al., 2004; González-Durán et al., 2008), but the theory that it is accumulated from the diet is supported by this study. The hypothesis would be that *S. purpureus* living on bare sand increases the proportion of food of faunal origin, and therefore DHA, in its diet, which implies an energetic benefit that can be seen in growth and reproduction.

4.4. Food quality and good fit at the different locations: the role of HUFA

It has been demonstrated that several aquatic organisms grow better when provided with HUFAs (highly unsaturated fatty acids), especially EPA (C20:4*n*-6) and DHA (C22:6*n*-3), from direct sources. HUFAs are quantitative measures of food quality and the good fit of organisms in aquatic ecosystems (Brett and Müller-Navarra, 1997; Ikawa, 2004). They are important structural and physiological components of cell membranes and their concentrations in natural or artificial diets impact survival, growth, development of specific tissues, and reproductive performance (fecundity, egg production and hatchability, spawning, etc.) (Brett and Müller-Navarra, 1997; Weers and Gulati, 1997; Budge et al., 2002; Li et al., 2005; Hyne et al., 2009).

Food quality was analyzed with a FA analysis of the main algal and sediment samples. The results reveal an important food source at L4: the algae *O. volubilis* and *P. crispa* are rich in DHA (probably due to contribution of faunal epiphytic organisms). Also in this location the sediment was richer in EPA. A high gonadosomatic index and higher EPA value in the gonads indicate a good fit for the population of sea urchin in this area. These fact permits assume that in this area the omnivorous diet in urchins, based in higher quality of food source (both the sediment and algae) increase gonadal growth, as has been demonstrated in laboratory and in the field (Cook et al., 1998, 2000; Fernandez and Boudereseque, 2000; Hughes et al., 2005). Evidences exist on the importance of red soft algae beds as essential habitat to commercial species *Scorpaena notata* in shelf bottoms of Balearic Island, showing a better body condition in areas where these habitat exist (Ordines and Massutí, 2009). Moreover, in this area there is a higher proportion of small sea urchins. This can be explained by the different hydrodynamic conditions (recruitment), fishing pressure and the available food resources. There is no data to test these possible differences, but the algal composition and the quality of both the algae and sediment suggest that in this location the juvenile sea urchins could have better nutritional conditions for growth. Considerable research into the aquaculture of fish, molluscs and crustaceans has demonstrated that there are strong dietary demands for HUFA-rich diets. The larval stages are more dependent than adults because their high somatic growth rates cannot be satisfied by their FA conversion capacities (Albentosa et al., 1994; Kanazawa and Koshio, 1994; Coutteau et al., 1996; Sargent et al., 1999; Izquierdo et al., 2001).

The sea urchins of location with bare sand (L1) also showed a high proportion of DHA (C22:6*n*-3) in the gonads and a high gonadosomatic index. In previous paragraph it was hypothesized on a carnivorous diet to explain the good fit for the population of this location. However, small sizes were not found in this area. One possible explanation is that juvenile stages cannot capture faunal components in the sediment, selecting habitats where others food sources are possible. The ability to select particles has been demonstrated in *S. purpureus*, e.g. special granulometric features and particles with organic cover (Jangoux and Lawrence, 1982). There are other possible reasons, such as geographical differences in demographic composition or differences in the reproductive state of the population, but data on this issue is not available.

4.5. The role of *S. purpureus* on shelf bottoms of the Balearic Islands

The data on the high abundances of *S. purpureus* on soft bottoms with soft red algae in the Balearic Islands (Ordines et al., 2009) can be considered evidence of the importance of this habitat as a provider of detrital carbon. In this respect, *S. purpureus* plays a potentially important role in biogeochemical processes and ecosystem functioning not only on bare sand (as suggested by other authors) but even for deep sea algal communities in the Balearic Islands. Like the spatangoid studied here, burrowing species common in New Zealand belonging to the genus *Echinocardium* (infaunal, grazers/deposit feeders) have been found to dominate bioturbation processes and be positively related to primary production (Lohrer et al., 2004). Bioturbation activity increases sediment permeability, water content and oxygen content, which influences remineralization rates and nutrient fluxes (Mirza and Gray, 1981; Widdicombe and Austen, 1999; Lohrer et al., 2004, 2005; Granberg et al., 2005). Moreover, secretions, faecal pellet production and excretions play an important role in sediment fertilization (Herman et al., 1999; Osinga et al., 1997). The key role that *S. purpureus* and similar burrowing urchins play in ecological processes highlights the importance of mitigating anthropogenic impacts. This species have been shown to be highly vulnerable to fishing disturbances due to bottom impacting gears (Nilsson and Rosenberg, 1994, 2000; Thrush et al., 1998; Jennings et al., 2001).

5. Conclusions

The current study shows that the FA profile of the gonads of *S. purpureus* can be a useful trophic marker. The profile agrees well with the FA composition of the potential trophic resources (algae and sediment) and reveals spatial changes in relation to habitat features and available resources. *S. purpureus* diet reflected the local availability of phytodetritus, faunal detritus and microbial mats or a combination of all three sources. The omnivorous feeding behavior of this species suggests that phytodetritus found within algae beds is an important carbon source for this sea urchin in habitats with algae, and on bare sand the sea urchin has a more carnivorous diet. The soft red algae *O. volubilis* and *P. crispa* showed high levels of polyunsaturated FAs, indicative of food quality. In the areas where these algae dominated the sea urchins showed higher gonadal growth. This suggests that a mixed diet based on a higher quality food source (both the sediment and algae) best explains the good fit of the sea urchin populations, which has been demonstrated in other studies both in the laboratory and the field (Cook et al., 1998, 2000; Fernandez and Bouderesque, 2000; Hughes et al., 2005). The combined use of FA signatures, gonadal indices and growth rates could be a useful tool for identifying the good fit of benthic species and identifying essential habitats.

Acknowledgments

The authors wish to thank the crew of the *Cornide de Saavedra* and all those who took part in the MEDITS0509 survey. This survey was carried out within the BADEMECO project, financed by the IEO and EU (Data Collection Framework for the Common Fisheries Policy). The FAs analyses were partially funded by FatFish project (CTM2009-14362-C02-02), Ministerio de Ciencia e Innovación (Spanish Government).

References

Ackman, R.G., Tocher, C.S., McLachlan, J., 1968. Marine phytoplankter fatty acids. Journal of the Fisheries Research Board of Canada 25, 1603–1620.
Ackman, R.G., 1981. Algae as a source of edible lipids. In: Pryde, E.H., Princen, L.H., Mukherjee, K.D. (Eds.), New Sources of Fats and Oils. AOCS Monograph 9. American Oil Chemists' Society, Champaign, IL, pp. 189–220.
Aitchison, J., 1986. The Statistical Analysis of Compositional Data. Chapman and Hall, New York.
Ayling, A.L., 1978. The relation of food availability and food preferences to the field diet of an echinoid *Evechinus chloroticus* (Valenciennes). Journal of Experimental Marine Biology and Ecology 33, 223–235.
Albentosa, M., Labartaa, U., Pérez-Camachoa, A., Fernandez-Reiriza, M.J., Beirasa, R., 1994. Fatty acid composition of Venerupis pullastra spat fed on different microalgae diets. Comparative Biochemistry and Physiology Part A: Physiology 108 (4), 639–648.
Beddingfield, S.D., Mc Clintock, J.B., 1999. Food resource utilization in the sea urchin *Lytechinus variegates* in contrasting shallow-water microhabitats of Saint Joseph Bay, Florida. Gulf of Mexico Science 1, 27–34.
Bell, M.V., Hendersona, R.J., Sargent, J.R., 1986. The role of polyunsaturated fatty acids in fish. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 83 (4), 711–719.
Bell, M.V., Dirk, J.R., Kelly, M.S., 2001. Biosynthesis of eicosapentaenoic acid in the sea urchin *Psammechinus miliaris*. Lipids 36 (1), 79–82.
Brett, M.T., Müller-Navarra, D., 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38, 483–499.
Brigmon, R.L., Ridder, C., 1998. Symbiotic relationship of *Thiothrix* spp. with an echinoderm. Applied and Environmental Microbiology 64 (9), 3491–3495.
Buchanan, J.B., Brown, B.E., Coombs, T.L., Pirie, B.J.S., Allen, J.A., 1980. The accumulation of ferric iron in the guts of some spatangoid echinoderms. Journal of Marine Biology 60, 631–640.
Budge, S.M., Iverson, S.J., Don Bowen, W., Ackman, R.G., 2002. Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence. Canadian Journal of Fisheries and Aquatic Sciences 59, 886–898.
Budge, S.M., Iverson, S.J., Koopman, H.N., 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science 22, 759–801.
Carpenter, R.C., 1981. Grazing by *Diadema antillarum* and its effects on the benthic algal community. Journal of Marine Research 39, 749–765.
Castell, J.D., Kennedy, E.J., Robinson, S.M.C., Parsons, G.J., González-Durán, T.J., 2004. Effects of dietary lipids on the fatty acid composition and metabolism in juvenile green sea urchins (*Strongylocentrotus droebachiensis*). Aquaculture 242, 417–435.
Chiold, J., 1989. Species distribution of irregular echinoids. Biological Oceanography 6, 79–162.
Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. American Journal of Ecology 18, 117–143.
Cook, E.J., Bell, M.V., Black, K.D., Kelly, M.S., 2000. Fatty acid compositions of gonadal material and diets of the sea urchin, *Psammechinus miliaris*: trophic and nutritional implications. Journal of Experimental Marine Biology and Ecology 255, 261–274.
Cook, E.J., Kelly, M.S., Mckenzie, J.D., 1998. Somatic and gonadal growth of the sea urchin Psammechinus miliaris (Gmelin) fed artificial salmon feed compared with a macroalgal diet. Journal of Shellfish Research 17, 1549–1555.
Cook, E.J., Hughes, A.D., Orr, H., Kelly, M.S., Black, K.D., 2007. Influence of dietary protein on essential fatty acids in the gonadal tissue of the sea urchins *Psammechinus miliaris* and *Paracentrotus lividus* (Echinodermata). Aquaculture 273, 586–594.
Coutteau, P., Camara, M.R., Sorgeloos, P., 1996. The effect of different levels and sources of dietary phosphatidylcholine on the growth, survival, stress resistance, and fatty acid composition of postlarval Penaeus vannamei. Aquaculture 147 (3–4), 261–273.
Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology 46, 225–340.
Emson, R.H., Moore, P.G., 1998. Diet and gonad size in three populations of Echinus esculentus. In: Mooi, R., Telford, M. (Eds.). Balkema, Rotterdam, pp. 641–644. Proceedings of the 9th International Echinoderm Conference, San Francisco.
Fergurson, J.C., 1976. The annual cycle of fatty acid composition in a starfish. Comparative Biochemistry and Physiology 54, 249–252.

Fernandez, C., Boudouresque, C.F., 2000. Nutrition of the sea urchin Paracentrotus lividus (Echinodermata: Echinoidea) fed different artificial food. Marine Ecology Progress Series 204, 131–141.
Fernandez-Jover, D., López Jimenez, J.A., Sanchez, P., 2007. Changes in body condition and fatty acid composition of wild Mediterranean horse mackerel (*Trachurus mediterraneus*, Steindachner, 1868) associated to sea cage fish farms. Marine Environmental Research 63, 1–18.
Folch, J., Lees, N., Sloan-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipid. Journal of Biological Chemistry 226, 497–509.
Ghiold, J., 1989. Species distributions of irregular echinoids. Biological Oceanography 6, 79–162.
Giddings, G.G., Hill, L.H., 1975. The effect of treatment on lipid fractions and basic fatty acid of the muscle blue crab (*Callinectes sapidus*). Journal of Food Science 40, 1127–1129.
Gillian, F.T., Stoilov, I.L., Thompson, J.E., Hogg, R.W., Wilkinson, C.R., Djerassi, C., 1988. Fatty acids as biological markers for bacterial symbionts in sponges. Lipids 23, 1139–1145.
Ginger, M.L., Santos, V., Wolff, G.A., 2000. A preliminary investigation of the lipids of abyssal holothurians from the northeast Atlantic Ocean. Journal of the Marine Biological Association of the United Kingdom 80, 139–146.
González-Durán, E., Castell, J.D., Robinson, S.M.C., Blair, T.J., 2008. Effects of dietary lipids on the fatty acid composition and lipid metabolism of the green sea urchin Strongylocentrotus droebachiensis. Aquaculture 276 (1–4), 120–129.
Grahl-Nielsen, O., Andersen, M., Derocher, A.E., Lydersen, C., Wiig, Ø, Kovacs, K.M., 2003. Fatty acid composition of the adipose tissue of polar bears and of their prey: ringed seals, bearded seals and harp seals. Marine Ecology Progress Series 265, 275–282.
Granberg, M.E., Hansen, R., Selck, H., 2005. Relative importance of macrofaunal burrows for the microbial mineralization of pyrene in marine sediments: impact of macrofaunal species and organic matter quality. Marine Ecology Progress Series 288, 59–74.
Gunstone, F.D., Harwood, J.L., Padley, F.B., 1994. Occurrence and characteristics of oils and fats. In: The Lipid Handbook. Chapman and Hall, London, pp. 44–223.
Harrold, C., Reed, D.C., 1985. Food availability, sea urchin grazing, and kelp forest community structure. Ecology 66 (4), 1160–1169.
Herman, P., Middleburg, J., Vande-Koppel, J., Heip, C., 1999. Ecology of estuarine benthos. Advances in Ecological Research 29, 165–231.
Holme, N.A., 1966. The bottom fauna of the English Channel. Part II. Journal of the Marine Biological Association of the United Kingdom 46 (2), 401–493.
Howell, K.L., Pond, D.W., Billett, D.S.M., Tyler, P.A., 2003. Feeding ecology of deep-sea seastars (Echinodermata: Asteroidea): a fatty-acid biomarker approach. Marine Ecology Progress Series 255, 193–206.
Hughes, A.D., Catarinio, A.I., Kelly, M.S., Barnes, D.K.A., Black, K.D., 2005. Gonad fatty acids and trophic interactions of the echinoid *Psammechinus miliaris*. Marine Ecology Progress Series 305, 101–111.
Hyne, R.V., Sánchez-Bayo, F., Bryan, A.D., Johnston, E., Mann, R.M., 2009. Fatty acid composition of the estuarine amphipod, *Melita plumulosa* (Zeidler): link between diet and fecundity. Environmental Toxicology and Chemistry 28, 123–132.
Ikawa, M., 2004. Algal Polyunsaturated Fatty Acids and Effects on Plankton Ecology and Other Organisms, vol. 6. UNH Center for Freshwater Biology Research (2): pp. 17–44.
Isay, S.V., Busarova, N.G., 1984. Study on fatty acid composition of marine organisms- I. Unsaturated fatty acids of Japan Sea invertebrates. Comparative Biochemistry and Physiology 77 (4), 803–810.
Iverson, S., Field, C., Don Bowen, V., Blanchard, W., 2004. Quantitative fatty acids signature analysis: a new method of estimating predator diets. Ecological Monographs 74 (2), 211–235.
Izquierdo, M.S., 1996. Essential fatty acids requirements of culture marine fish larvae. Aquaculture Nutrition 2, 183–191.
Izquierdo, M.S., Tandler, A., Salhi, M., Kollovski, S., 2001. Influence of dietary polar lipids quantity and quality on ingestion and assimilation of labeled fatty acids by larval gilthead seabream. Aquaculture Nutrition 7, 153–160.
Jangoux, M., Lawrence, J.M., 1982. Echinoderm Nutrition. A.A. Balkema Press, Rotterdam.
Jennings, S., Pinnegar, J.K., Polunin, N.V.C., Warr, K.J., 2001. Impacts of trawling disturbance on the trophic structure of benthic invertebrate communities. Marine Ecology Progress Series 213, 127–142.
Käkelä, R., Käkelä, A., Kahle, S., Becker, P.H., Kelly-Robert, A., Furness, W., 2005. Fatty acid signatures in plasma of captive herring gulls as indicators of demersal or pelagic fish diet. Marine Ecology Progress Series 293, 191–200.
Kanazawa, K., 1992. Adaptation of test shape for burrowing and locomotion in spatangoid echinoids. Paleontology 35 (4), 733–750.
Kanazawa, A., Koshio, S., 1994. Lipid nutrition of the spiny lobster Panulirus japonicas (decapoda, lalinuridae)- A review. Crustaceana 67, 226–232.
Kayama, M., Araki, S., Sato, S., 1989. Lipids of marine plants. In: Ackman, R.G. (Ed.), Marine Biogenic Lipids, Fats and Oils, vol. 2. CRC Press, Boca Raton, Florida, pp. 3–48.
Kharlamenko, V.I., Zhukova, N.V., Khotimchenko, S.V., Svetashev, V.I., Kamenvev, G.M., 1995. Fatty acids as markers of food sources in a shallow water hydrothermal ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands). Marine Ecology Progress Series 120, 231–241.
Kharlamenko, V.I., Kiyashko, S.I., Imbs, A.B., Vyshkvartzev, D.I., 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. Marine Ecology Progress Series 220, 103–117.
Khotimchenko, S.V., Vaskowsky, V.E., Titlyanova, T.V., 2002. Fatty Acids of Marine Algae from the Pacific Coast of North California. Botanica Marina 45, 17–22.
Lawrence, J.M., 1975. On the relationships between marine plants and sea-urchins. Oceanography and Marine Biology Annual Review 13, 213–286.
Lawrence, J.M., 2007. Edible Sea Urchins: Biology and Ecology. Developments in Aquaculture and Fisheries Sciences, vol. 37. Elsevier, Oxford, UK.
Lee, R.F., Nevenzel, J.C., Paffenhofer, G.A., 1971. Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Marine Biology 9, 99–108.
Lenningher, A., 1984. Principios de bioquímica. Omega, Barcelona, p. 1013.
Lewis, R.W., 1967. Fatty acid composition of some marine animals from various depths. Journal of the Fisheries Research Board of Canada 24, 1101–1115.
Li, Q., Wang, M., Tan, L., Wang, C., Ma, J., Li, N., Li, Y., Xu, G., Li, G., 2005. Docosahexaenoic acid changes lipid composition and IL-2 receptor signaling in membrane rafts. Journal of Lipid Research 46, 1904–1913.
Liu, H., Kelly, M.S., Cook, E.J., Black, K., Orr, H., Xin Zhu, J., Lin Dong, S., 2007a. The effect of diet type on growth and fatty-acid composition of sea urchin larvae, I. *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata). Aquaculture 264, 247–262.
Liu, H., Kelly, M.S., Cook, E.J., Black, K., Orr, H., Xin Zhu, J., Lin Dong, S., 2007b. The effect of diet type on growth and fatty acid composition of the sea urchin larvae, II. *Psammechinus miliaris* (Gmelin). Aquaculture 264, 263–278.
Liyana-Pathiranaa, Ch., Shahidia, F., Whittick, A., 2002. The effect of an artificial diet on the biochemical composition of the gonads of the sea urchin (*Strongylocentrotus droebachiensis*). Food Chemistry 79, 461–472.
Lohrer, A.M., Thrush, S.F., Gibbs, M.M., 2004. Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 431, 1092–1095.
Lohrer, A.M., Thrush, S.F., Hunt, L., Hancock, N., Lundquist, C.J., 2005. Rapid reworking of subtidal sediments by burrowing spatangoid urchins. Journal of Experimental Marine Biology and Ecology 321 (2), 155–169.
Lohrer, A.M., Chiaroni, L.D., Hewitt, J.E., Thrush, S.F., 2008. Biogenic disturbance determines invasion success in a subtidal soft-sediment system. Ecology 89 (5), 1299–1307.
Mai, K., Mercer, J.P., Donlon, J., 1996. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino.V. The role of polyunsaturated fatty acids of macroalgae in abalone nutrition. Aquaculture 139, 77–89.
Mansour, M.P., Volkman, J.K., Jackson, A.E., Blackburn, S.I., 1999. The fatty acid and sterol composition of five marine dinoflagellates. Journal of Phycology 35, 710–720.
Mirza, F.B., Gray, J.S., 1981. The fauna of benthic sediments from the organically enriched Oslofjord, Norway. Journal of Experimental Marine Biology and Ecology 54, 181–207.
Nelson, M.M., Phleger, C.F., Nichols, P.D., 2002. Seasonal lipid composition in macroalgae of the northeastern Pacific Ocean. Botanica Marina 45, 58–65.
Nichols, P.D., Johns, R.B., Klumpp, D.W., 1982. Study of food-chains in seagrass communities. 1. Lipid components of the seagrasses *Posidonia australis* and *Heterozostera tasmanica* as indicators of carbon source. Phytochemistry 21, 1613–1621.
Nilsson, H.C., Rosenberg, R., 1994. Hypoxic response of two marine benthlc communities. Marine Ecology Progress Series 115, 209–217.
Nilsson, H.C., Rosenberg, R., 2000. Succession in marine benthic habitats and fauna in response to oxygen deficiency: analyzed by sediment profile-imaging and by grab samples. Marine Ecology Progress Series 197, 139–149.
Ordines, F., Massutí, E., 2009. Relationships between macro-epibenthic communities and fish on the shelf grounds of the western Mediterranean. Aquatic Conservation: Marine and Freshwater Ecosystems 14 (4), 370–383.
Ordines, F., Quetglas, A., Massutí, E., Moranta, J., 2009. Habitat preference and life history of the red scorpion fish, Scorpaena notata, in the Mediterranean. Estuarine. Coastal and Shelf Science 85, 537–546.
Osinga, R., Kop, A.J., Malschaert, J.F.P., van Duyl, F.C., 1997. Effects of the sea urchin *Echinocardium cordatum* on bacterial production and carbon flow in experimental benthic systems under increasing organic loading. Journal of Sea Research 37, 109–121.
Paradis, M., Ackman, R.G., 1977. Potential for employing the distribution of anomalous non-methylene-interrupted dienoic fatty acids in several marine invertebrates as part of food web studies. Lipids 12 (2), 170–176.
Parkes, R.J., 1987. Analysis of microbial communities within sediments using biomarkers. In: Ecology of Microbial Communities. SGM 41. Cambridge University Press, Cambridge.
Pond, D., Allen, C.E., Bell, M.V., van Dover, C.L., Fallick, A.E., Dixon, D.R., Sargent, J.R., 2002. On the origins of long-chain, polyunsaturated fatty acids in the hydrothermal vent worms, *Ridgea piscesae* and *Protis hydrothermica*. Marine Ecology Progress Series 142, 175–184.
Romashina, N.A., 1983. Marine invertebrates as a source of eicosapentaenoic and other polyenoic acids. Biologia Moray 1, 66–68.
Sargent, J.R., Henderson, R.J., 1995. Marine (n-3) polyunsaturated fatty acids. In: Hamilton, R.J. (Ed.), Developments in Oils and Fats. Blackie Academic and Professional, London, pp. 32–65.
Sargent, J.R., Whittle, K.J., 1981. Lipids and hydrocarbons in the marine food web. In: Longhurst, A.R. (Ed.), Analysis of Marine Ecosystems. Academic Press, London, pp. 491–533.
Sargent, J.R., Parkes, R.J., Mueller-Harvey, I., Henderson, R.J., 1987. Lipid biomarkers in marine ecology. In: Sleigh, M.A. (Ed.), Microbes in the Sea. Ellis Horwood, Chichester, pp. 119–138.

Sargent, J.R., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191–199.
Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Elsevier, USA, pp. 181–257.
Stoffel, W., Chu, F., Edward, H., 1959. Analysis of long-chain fatty acids by gas–liquid chromatography. Micromethod for preparation of methyl esters. Analytical Chemistry 31, 307–308.
Stryer, L., 1995. Biochemistry, fourth ed. WH Freeman, New York.
Takagi, T., Eaton, C.A., Ackman, R.G., 1980. Distribution of fatty acids in lipids of the common Atlantic sea urchin *Strongylocentrotus droebachiensis*. Canadian Journal of Fisheries and Aquatic Sciences 37, 195–202.
Temara, A., De Ridder, C., Raisin, M., 1991. Presence of an essential polyunsaturated fatty acids in intradigestive bacterial symbionts of a deposit-feeder echinoid (Echinodermata). Comparative Biochemistry and Physiology 100, 503–505.
Temara, A., De Ridder, C., Kuenen, J.G., Robertson, L.A., 1993. Sulfide-oxidizing bacteria in the burrowing echinoid, *Echinocardium cordatum* (Echinodermata). Marine Biology 115, 179–185.
Ter Braak, C.J.F., Šmilauer, P., 2002. CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (Version 4.5). Microcomputer Power, Ithaca, New York. http://www.canoco.com p. 500.
Thrush, S.F., Dayton, P.K., 2002. Disturbance to marine benthic habitats by trawling and dredging – implications for marine biodiversity. Annual Review of Ecology and Systematics 33, 449–473.
Thrush, S.F., Hewitt, J.E., Cummings, V.J., Dayton, P.K., Cryer, M., Turner, S.J., Funnell, G.A., Budd, R.G., Milburn, C.J., Wilkinson, M.R., 1998. Disturbance of the marine benthic habitat for commercial fishing: impacts at the scale of the fishery. Ecological Applications 8, 866–897.
Vadas, R.L., 1977. Preferential feeding: an optimization strategy in sea urchins. Ecological Monographs 47, 337–371.
Vashappilly, R., Chen, F., 1998. Heterotrophic production potential of omega-3 polyunsaturated fatty acids by microalgae and algae-like mico-organisms. Botanica Marina 41, 553–558.
Verlaque, M., 1987. Relations entre *Paracentrotus lividus* (Lamarck) et le phytobenthos de Méditerranée occidentale. In: Boudouresque, C.F. (Ed.), International sur *Paracentrotus lividus* et les oursins comestibles. GIS Posidonie Publications, pp. 5–36.
Verreth, J., Coppoolse, J., Segner, H.T., 1994. The effect of low HUFA- and high HUFA-enriched Artemia, fed at different feeding levels, on growth, survival, tissue fatty acids and liver histology of Clarias gariepinus larvae. Aquaculture 126, 137–150.
Volkman, J.K., Bavor, H.J., Gillan, F.T., Johns, R.B., Perry, G.J., 1980. Microbial lipids of an intertidal sediment. 1. Fatty acids and hydrocarbons. Geochimica and Cosmochimica Acta 44, 1133–1143.
Weers, P.M.M., Gulati, R.D., 1997. Effect of addition of polyunsaturated fatty acids to the diet on growth and fecundity of *Daphnia galeata*. Freshwater Biology 38, 721–729.
Widdicombe, S., Austen, M.C., 1999. Mesocosm investigation into the effects of bioturbation on the diversity and structure of a subtidal macrobenthic community. Marine Ecology Progress Series 189, 181–193.
Widdicombe, S., Austen, M.C., Kendall, M.A., Olsgard, F., Schaanning, M.T., Dashfield, S.L., Needham, H.R., 2004. Importance of bioturbators for biodiversity maintenance: indirect effects of fishing disturbance. Marine Ecology Progress Series 275, 1–10.
Yano, Y., Nakayama, A., Yoshida, K., 1997. Distribution of polyunsaturated fatty acids in bacteria present in intestines of deep-sea fish and shallow-sea poikilothermic animals. Applied and Environmental Microbiology 63, 2572–2577.
Zar, J.H., 1996. Bioestatistical analisis. Prentice Hall, USA, p. 662.
Řezanka, T., Sigler, K., 2009. Odd-numbered very-long-chain fatty acids from the microbial, animal and plant kingdoms. Progress in Lipid Research 48 (3–4), 206–238.