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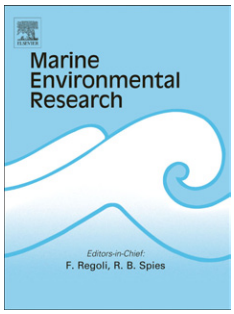

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Trophic ecology of the sea urchin *Spatangus purpureus* elucidated from gonad fatty acids composition analysis

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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* agrees 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 behaviour; 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.

Key words: sea urchins, fatty acids, feeding, diet, soft red algae, sandy bottoms, sediment, detritus, bioturbation, deposit feeder, Mediterranean Sea.

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 macro-algae (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 (Thush 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 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, 1986). 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 Mcclintock, 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-Pathirana 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 *Spatangus 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., *Osmundaria volubilis* and *Phyllophora 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 x 3 sites x 5 replicates). Three subsamples of the three dominant soft algae, *Osmundaria volubilis, Peyssonnelia* spp. and *Phyllophora crispa*, were also obtained from the beam-trawl samples at three different sites of vegetated locations (5-6 g) (3 species x 3 sites x 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 x 3 sites x 2 replicates). All samples were frozen in glass tubes with Teflon-lined screw caps, and conserved at -80ºC until FA analysis in the laboratory.

135

136 *2.3. Laboratory methods*

137

138 After individual sample/tissue homogenization, the FA composition of the total lipid
139 fraction was determined by fat extraction following the method of Folch et al. (1957),
140 with a mixture of chloroform and methanol (1:1 proportion for the first extraction and
141 2:1 proportion for the second one). Fatty acid methyl ester (FAME) samples were
142 analyzed according to the method of Stoffel et al. (1959) by gas-liquid chromatography
143 using a SP^TM 2560 flexible fused silica capillary column (100-m long, internal
144 diameter of 0.25-mm and film thickness of 0.20 μm; SUPELCO) in a Hewlett-Packard
145 5890 gas chromatograph. The oven temperature of the gas chromatograph was
146 programmed for 5 min at an initial temperature of 140ºC, and increased at a rate of 4ºC
147 per min to 230ºC, further increased at a rate of 1ºC per min to 240ºC and then held at
148 that temperature for 6 min. The injector and flame ionization detector were set a 250ºC.
149 Helium was used as carrier gas at a pressure of 290 kPa, and peaks were identified by
150 comparing their retention times with appropriate FAME standards purchased from the
151 Sigma Chemical Company (St. Louis, MO, USA). Individual FA concentrations were
152 expressed as percentages of the total content.

153

154 *2.4. Statistical analysis*

155

156 Multivariate analysis was carried out using PRIMER v5 (Plymouth Routines In
157 Multivariate Ecological Research) in order to compare the FA profiles of different
158 sample groups (gonads, algae and sediment) and examine the differences within these

159 sample groups. The data were converted into similarity matrices using a *Bray-Curtis*
160 resemblance measure. Permutation-based analysis of similarity (ANOSIM) was used to
161 examine differences in fatty acid profiles across the factors location (for gonads and
162 sediment) and species (for algae). SIMPER analysis (PRIMER 6. software) was used to
163 investigate the similarities and dissimilarities within and between sample groups and the
164 main fatty acids contributing to these (Clarke, 1993). Redundancy Analysis (RDA) was
165 used to test how much of the variance in the multivariate analysis of the FAs in sea-
166 urchin gonads (species variables) can be explained by the following factors
167 (environmental variables): location, sea urchin size (S) and gonadosomatic index
168 (GSI=gonad weight/body size). RDA were performed using the software CANOCO
169 (version 4.5) following the procedure established for compositional data (e.g.
170 percentage) using the log-ratio analysis centred both by samples and individual FAs
171 (Aitchinson, 1986; ter Braack and Smilauer 2002).
172
173 Analysis of variance (ANOVA) was used to test whether the biological parameters of
174 sea urchins (size, gonad weight and GSI by size class), the main FAs and other
175 biochemical parameters (n-3/n-6 ratio, C18:1n-9/C18:1n-7 ratio, ARA/EPA) varied
176 among sea urchin gonads collected at different locations. Biological parameters were
177 analyzed by an unbalanced 1way-ANOVA (factor location with 4 treatments) with n=
178 25-50 individuals. The linear model of FA analysis incorporates two factors: location
179 (fixed, with 4 treatments) and site (random and nested, with 3 treatments). Five
180 replicates were carried out. The FA data are presented as percentages, which require
181 arcsine transformation to produce a normally distributed data set with homogeneous
182 variances (Zar, 1996). Cochran's test was used before the analysis to check for
183 homogeneity of variances in different data populations. When Cochran's test showed

184 significant differences, the significance level of the ANOVA was set at 0.01. When the
185 ANOVA detected significant differences for any factor, the Student-Newman-Keuls
186 (SNK) test was applied.

187

188 **3. Results**

189

190 *3.1. Algal and faunal location characteristics*

191 The four locations selected for this study had sandy bottoms with different algal and
192 faunal communities (Table 1). Location 1 (L1) was typified by bare sandy bottoms
193 without algae. Location 2 (L2) was a vegetated substrate with a high biomass of
194 rhodoliths and other rhodoficies, especially *Osmundaria volubilis*. Location 3 (L3) was
195 an algal bed dominated by *Peyssonnelia* spp. and rhodoliths. Location 4 (L4) also had
196 high concentrations of rhodoliths and soft algae, including *Phyllophora crispa*, but had
197 a lower algal biomass than L2 and L3. The faunal abundance and biomass were higher
198 in L3 and L4. Sea urchins reached a maximum mean value of 2336.00 ind/ha (= 0.2
199 ind/m^2) in L4. The density of *S. purpureus* was intermediate in L2 and L3 compared
200 with the other locations, and it was very low in L1 (54.41 ind/ha= 0.0054 ind/m^2) (Table
201 1).

202

203 *3.2. Biological parameters of Spatangus purpureus*

204

205 The mean size of *S. purpureus* was 92.20 mm, showing differences between locations
206 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 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

230 percentages, by n-9 (15.50 ± 1.54%), n-3 (10.57 ± 2.25%) and n-6 FAs (8.14 ± 1.27%)
231 (Table 3).

232

233 The main FA components in the *S. purpureus* gonads were C20:4n-6 (17.46 ± 0.99%),
234 C16:0 (16.32 ± 0.22%), C20:5n-3 (15.19 ±0.63%) and C16:1n-7 (13.52 ± 0.66%)
235 (Table 3). However, the ANOSIM test showed that the FA profiles were slightly
236 different between the 4 locations except for L2 and L3 (R=0.275), and was especially
237 significant for L1 and L4 (R=0.849) (Table 4). The main FAs were the most important
238 for the similarity between samples in all cases, but the order of importance of their
239 contribution changed with the location (Table 5). The dissimilarity was mainly due to
240 the proportions of C16:1n-7, C20:4n-6 and C20:5n-3, and secondarily due to C22:6 n-3
241 and C14:0, which marked the differences between L1 and the rest of the locations
242 (SIMPER, Table 4). The main FAs in algae were C16:0 (33.70 ± 1.52%), C20:4 n-6
243 (17.50 ± 0.86%) and C20:5 n-3 (9.00 ± 0.86%) (Table 3); however, the FA profile
244 changed between species (R=0.691), and the differences between *O. volubilis* and
245 *Peyssonnelia* sp. (R=0.952) were the most significant. The dissimilarity between
246 samples was explained by these FAs and C22:6 n-3. This FA was more important in *O.*
247 *volubilis* and *P. crispa* than in *Peyssonnelia* sp., while in this algae C16:0 was higher
248 (Table 3). Moreover, C16:0 was the main component in the FA composition of
249 sediment (26.74 ± 0.69%), followed by C18:1n-9 (14.40 ± 1.12%) and C16:1 n-7 (13.42
250 ± 1.01%) (Table 3). In this case, the differences between locations were not significant
251 due to the high variation between replicates (R=0.174, Table 4), except for L1 and L4
252 (R=6.626; Table 4). The dissimilarity in this case was due to variation in C18:1 n-9,

253 C16:1 n-7 and C20:4 n-6. The two first FAs were highest in L1, while the third in L4
254 (Table 3).

255

256 *3.4. Changes in fatty acid composition in gonads in relation to location and biological*
257 *parameters*

258

259 The RDA results showed that two explanatory variables in the model were significant:
260 location and gonadosomatic index (GSI). Location explained 64.3% of the variance
261 (p=0.002) (Fig. 4, Table 6). The largest proportion of the variance on the correlation
262 with the first PCA components was explained by the algae biomass of the sampled
263 locations, in a gradient from locations with less algae biomass (L1 and L4) to locations
264 with more algal biomass (L2 and L3). However, this variance was also explained by the
265 different FA profile of L4 samples. Particular FAs contributes to variance between
266 locations (Fig. 4). The univariate analysis (ANOVA) applied to FAs helped to define
267 the changes in the FA proportions in relation to location (Fig. 5). The proportion of FAs
268 C14:0 and C22:6 n-3 was significantly higher in the gonads extracted from sea urchins
269 collected at L1 (sand) . C16:1 n-7, C18:1 n-9 and C20:5 n-3 were more abundant in the
270 gonads of sea urchins from L4, a location with a lower algal biomass, while C20:4 n-6
271 was less abundant. There was a lower proportion of C18:1n-7 at L2 (rhodoliths and soft
272 algae, such as *Osmundaria volubilis*). The FAs C15:0, C17:0, C20:0, C17: 1n-7, C18:2
273 n-6, C22:2 n-6 and C24:1 n-9 were also relevant for the variation in the FA profile in
274 relation to location (Fig. 4), but they represented only a small proportion of the FA
275 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). 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 n3/n6 also showed significant differences in relation to location (Fig. 5). The ratio C18:1 (n9/n7) was higher at L4 (bare sand) and L2 (rhodoliths and soft algae such as *Osmundaria 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

299 signature varied between locations, and reflected the availability of food resources and
300 possible dietary adaptations (Ginger et al., 2000; Sargent et al., 1999, 2002). Another
301 factor affecting the FA profile is gonadal development (Cook et al., 2007). The results
302 evidences that *S. purpureus* feeds on a wide range of potential food (phytodetritus,
303 faunal detritus and bacterial mats), which is clearly indicative of an omnivorous diet.

304

305 *4.1. FA composition of S. purpureus and potential food sources*

306

307 The FA profiles of *S. purpureus* gonads show large proportions of palmitic (C16:00),
308 arachidonic (C20:4n-6), eicosapentaenoic (C20:5n-3) and vaccenic (C16:1n-7) acids.
309 The pattern was similar in algae, except for vaccenic acid, which was common in the
310 sediment samples. The FA composition in *S. purpureus* can be interpreted as a
311 combination of fatty acids of vegetal origin (algal detritus), animal origin (infaunal and
312 faunal detritus) and others related to sediment (organic matter, bacteria). In general,
313 PUFAs explain a vegetal or animal origin in the diet, being predominant components in
314 the lipids of higher plants and animals. However, another possible origin is possible and
315 is discussed in the next sections. Saturated and monounsaturated FA dominated in
316 sediment samples, explained for the FA composition of bacteria, where PUFA are
317 absent (Lenninger, 1984). Saturated and monounsaturated have also been defined as
318 major components in algae (Ackman, 1981), but in the current work also arachidonic
319 and eicosapentaenoic have been defined as the main FA in algae, consistent with others
320 studies on red algae (Khotimchenko et al. 2002; Nelson et al. 2002). Diverse PUFA
321 have been identified as qualitative markers of fatty acids of vegetal origin in the trophic
322 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 (C16:1n-7) and oleic acid (C18:1n-9), both precursors in the formation of polyunsatured 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 (C18:3n-3), which belong to the group of essential FAs (Lenningher, 1986). 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 herviborous 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).

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, 2204) and are

predominant components in the lipids of higher plants and animals. Eicosapentaenoic acid (C20:5n-3) and arachidonic acid (C20:4n-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, 1986). 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 (C20:5n-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 (C20:4n-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

371 analyzed in the current work, suggesting that probably it is produced by the urchins
372 themselves.

373

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

392

393 *4.3. Trophic markers of bacterial input and carnivorous diet*

394

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., 1997; 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, 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; Ferguson, 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 et al., 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 el 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

443 n-3), from direct sources. HUFAs are quantitative measures of food quality and the
444 good fit of organisms in aquatic ecosystems (Brett and Müller-Navarra, 1997; Ikawa,
445 2004). They are important structural and physiological components of cell membranes
446 and their concentrations in natural or artificial diets impact survival, growth,
447 development of specific tissues, and reproductive performance (fecundity, egg
448 production and hatchability, spawning, etc.) (Brett and Müller-Navarra, 1997; Weers
449 and Gulati, 1997; Budge et al., 2002; Li et al., 2005; Hyne et al. 2009).
450
451 Food quality was analyzed with a FA analysis of the main algal and sediment samples.
452 The results reveal an important food source at L4: the algae *Osmundaria volubilis* and
453 *Phyllophora crispa* are rich in DHA (probably due to contribution of faunal epiphytic
454 organisms). Also in this location the sediment was richer in EPA. A high
455 gonadosomatic index and higher EPA value in the gonads indicate a good fit for the
456 population of sea-urchin in this area. These fact permits assume that in this area the
457 omnivorous diet in urchins, based in higher quality of food source (both the sediment
458 and algae) increase gonadal growth, as has been demonstrated in laboratory and in the
459 field (Cook et al. 1998, Fernandez and Bouderesque, 2000; Cook et al. 2000; Hughes et
460 al. 2005). Evidences exist on the importance of red soft algae beds as essential habitat to
461 commercial species *S. notata* in shelf bottoms of Balearic Island, showing a better body
462 condition in areas where these habitat exist (Ordines and Massuti, 2009). Moreover, in
463 this area there is a higher proportion of small sea urchins. This can be explained by the
464 different hydrodynamic conditions (recruitment), fishing pressure and the available food
465 resources. There is no data to test these possible differences, but the algal composition
466 and the quality of both the algae and sediment suggest that in this location the juvenile
467 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 carnivorus 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 *Spatangus 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.

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

493 species common in New Zealand belonging to the genus *Echinocardium* (infaunal,
494 grazers/deposit feeders) have been found to dominate bioturbation processes and be
495 positively related to primary production (Lohrer et al., 2004). Bioturbation activity
496 increases sediment permeability, water content and oxygen content, which influences
497 remineralization rates and nutrient fluxes (Mirza and Gray, 1981; Widdicombe and
498 Austen, 1999; Lohrer et al., 2004, 2005; Granberg et al., 2005). Moreover, secretions,
499 faecal pellet production and excretions play an important role in sediment fertilization
500 (Herman et al., 1999; Osinga et al., 1997). The key role that *S. purpureus* and similar
501 burrowing urchins play in ecological processes highlights the importance of mitigating
502 anthropogenic impacts. This species have been shown to be highly vulnerable to fishing
503 disturbances due to bottom impacting gears (Nilsson and Rosenberg, 1994, 2000;
504 Thrush et al., 1998, Jennings et al., 2001).

505

506 **5. Conclusions**

507

508 The current study shows that the FA profile of the gonads of *S. purpureus* can be a
509 useful trophic marker. The profile agrees well with the FA composition of the potential
510 trophic resources (algae and sediment) and reveals spatial changes in relation to habitat
511 features and available resources. *S. purpureus* diet reflected the local availability of
512 phytodetritus, faunal detritus and microbial mats or a combination of all three sources.
513 The omnivorous feeding behaviour of this species suggests that phytodetritus found
514 within algae beds is an important carbon source for this sea urchin in habitats with
515 algae, and on bare sand the sea urchin has a more carnivorous diet. The soft red algae
516 *Osmusdaria volubilis* and *Phyllophora 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, Fernandez and Bouderesque, 2000; Cook et al. 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.

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Figure legends

Figure 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.

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

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

815

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

820

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

824

825

Table 1

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)
L1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
L2	1585.14 ± 316.26	1576.72 ± 316.53	8.40 ± 2.02	0.00 ± 0.00
L3	8590.14 ± 1866.50	1344.64 ± 32.61	337.62 ± 32.61	6752.49 ± 652.16
L4	617.32 ± 42.07	361.65 ± 29.00	255.67 ± 39.05	0.00 ± 0.00

	Abundance fauna (ind/ha)	Biomass fauna (kg/ha)	*S. purpureus* (ind/ha)
L1	4467.86 ± 1797.37	49.03 ± 17.90	54.41 ± 32.81
L2	4042.90 ± 1151.84	134.53 ± 6.49	445.80 ± 148.50
L3	200428.87 ± 106193.27	922.40 ± 398.11	696.00 ± 264.00
L4	42262.70 ± 18956.57	518.57 ± 414.35	2336.00 ± 1877.00

Table 2

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 weigth (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

Table 3

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	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	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	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
C18:1n-9/C18:1n-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&L4	0.554	0.001	0.08	n.s.			

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

Gonads	All S=85.49%	L1 S=93.71%	L2 S=86.08%	L3 S=87.54%	L4 S=89.00%
C20:4 n-6	18.02	15.89	17.15	20.44	10.27
C16:0	17.83	14.96	19.3	18.33	16.72
C20:5 n-3	14.37	14.71	14.59	12.53	22.03
C16:1 n-7	13.09	11.74	15.51	12.1	20.08

Algae	All S=80.60%	Os S=87.63%	Pe S=86.86%	Ph S=84.52%
C16:0	36.30	29.54	46.1	34.85
C20:4 n-6	18.51	18.37	14.73	19.73
C20:5 n-3	8.14	14.27	6.39	6.26

Sediment	All S=73.91%	L1 S=84.53%	L2 S=75.26%	L3 S=62.22%	L4 S=81.42%
C16:0	31.31	29.31	32.27	28.87	31.56
C18:1 n-9	14.88	21.41	12.98	14.67	10.48
C16:1 n-7	13.27	10.2	17.16	10.54	15.44

Dissimilarity

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%
C16:1 n-7	13.01	11.2	17.96	9.89	13.94
C22:6 n-3	11.39	9.64	7.17		
C20:4 n-6	10.27	13.29	14.05	18.13	22.1
C14:0	9.47	10.32	8.18		
C20:5 n-3	8.74	11.64	16.9	20.34	22.73

Algae	Os&Pe S=24.95%	Os & Ph S=19.00%	Pe & Ph S=21.25%
C16:0	33.05	12.51	28.2
C20:5 n-3	16.19	21.04	
C22:6 n-3	9.92	15.56	16.7
C20:4 n-6	8.68	13.23	15.6

Sediment	L1&L4 S=24.21%
C18:1 n-9	19.17
C16:1 n-7	13.64
C20:4 n-6	8.21

Table 6

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

Table 7

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; 1995. Vashappilly and Chen, 1998; Mansour et al., 1999; Hughes et al. 2005
		Zooplankton	Kharlamenko et al., 2001
		Carnivorous feeding	Tagaki et al., 1986; 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., 2000; ;
		Algae	Nichols et al., 1982; Kayama et al., 1989; Nelson et al. 2002
C18:4 (n-3)	Stearidonic	Herbivorous	Sargent et al., 1983; 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 ; Gilian 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., 2003

Figure 1

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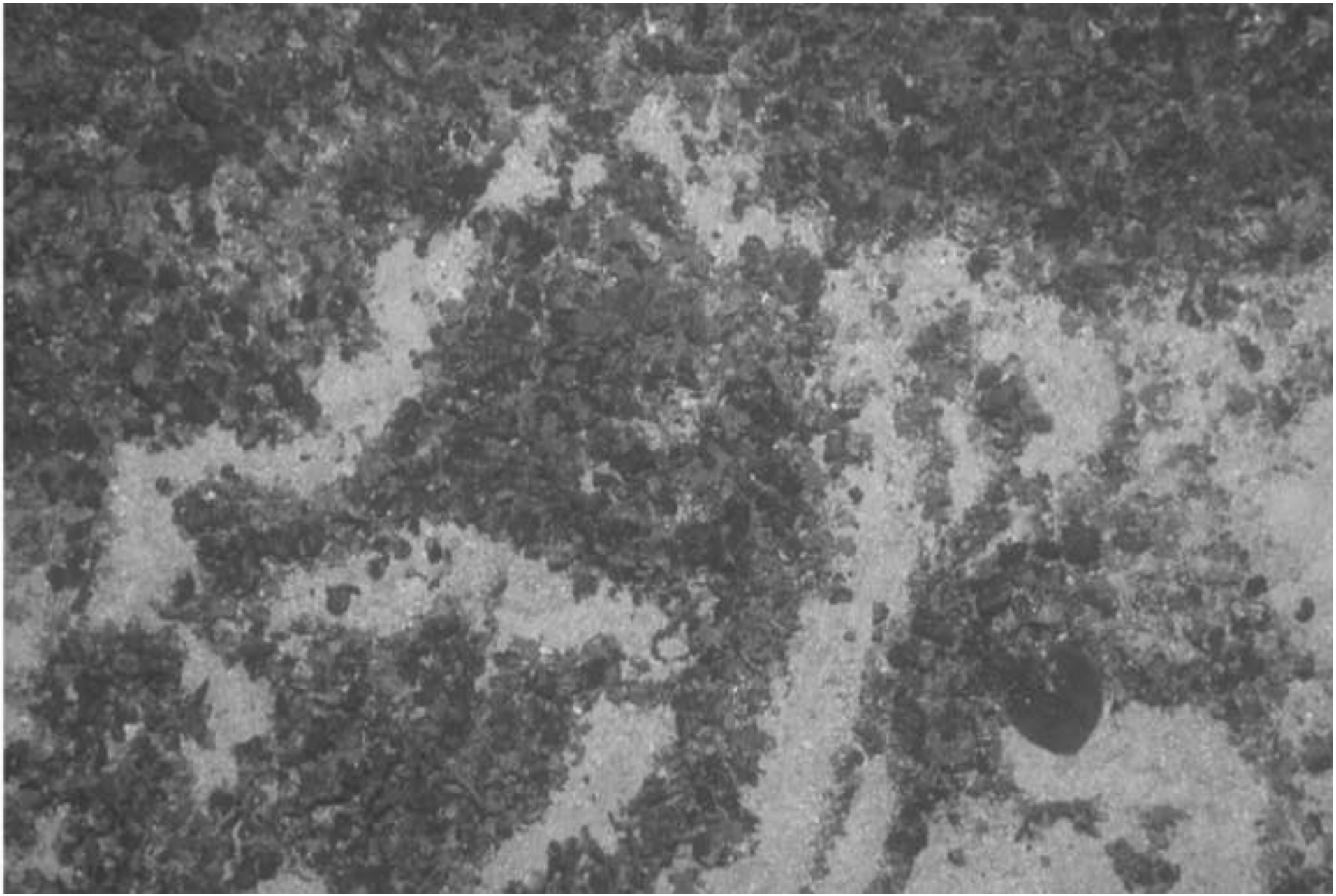

Figure 2

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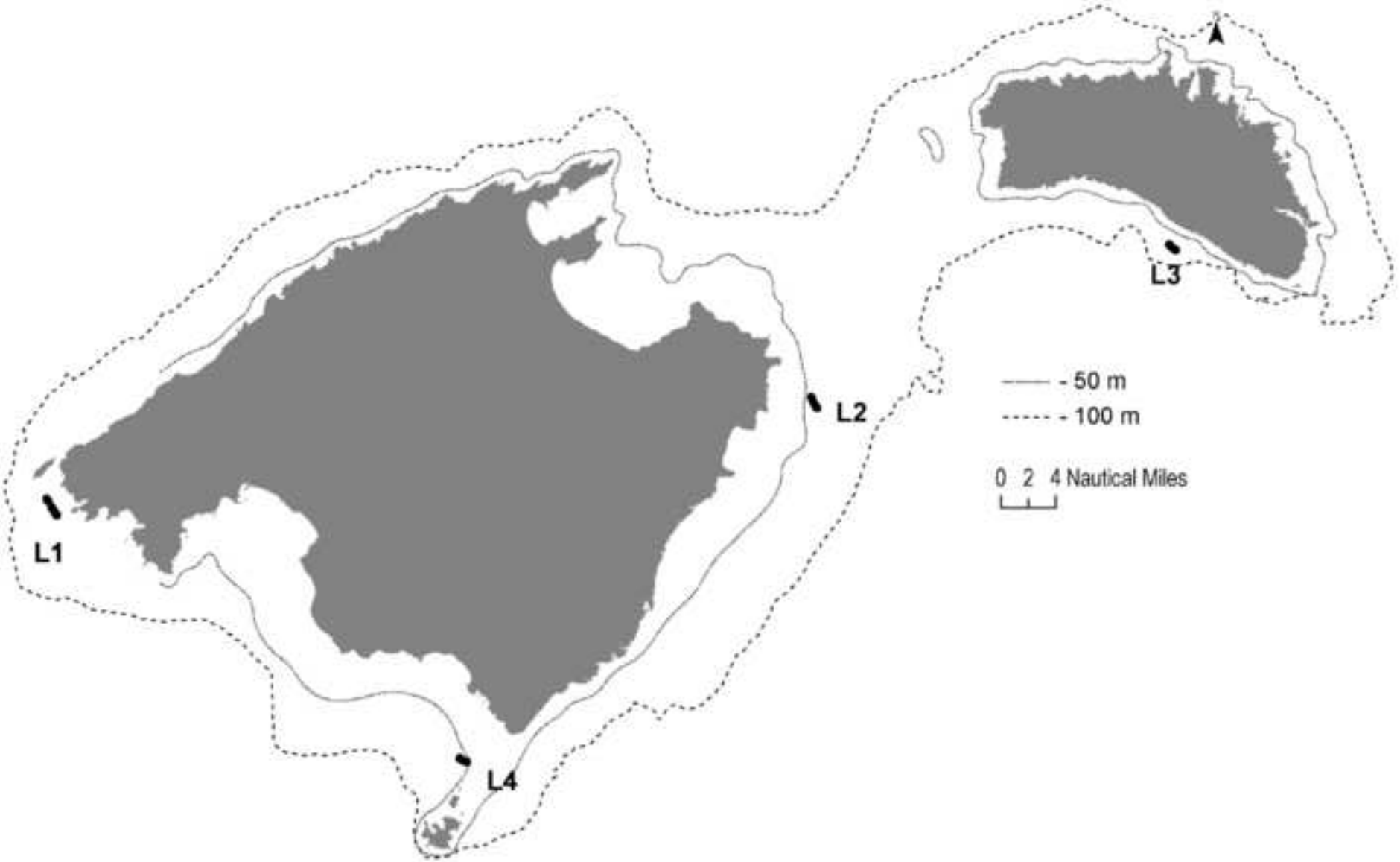

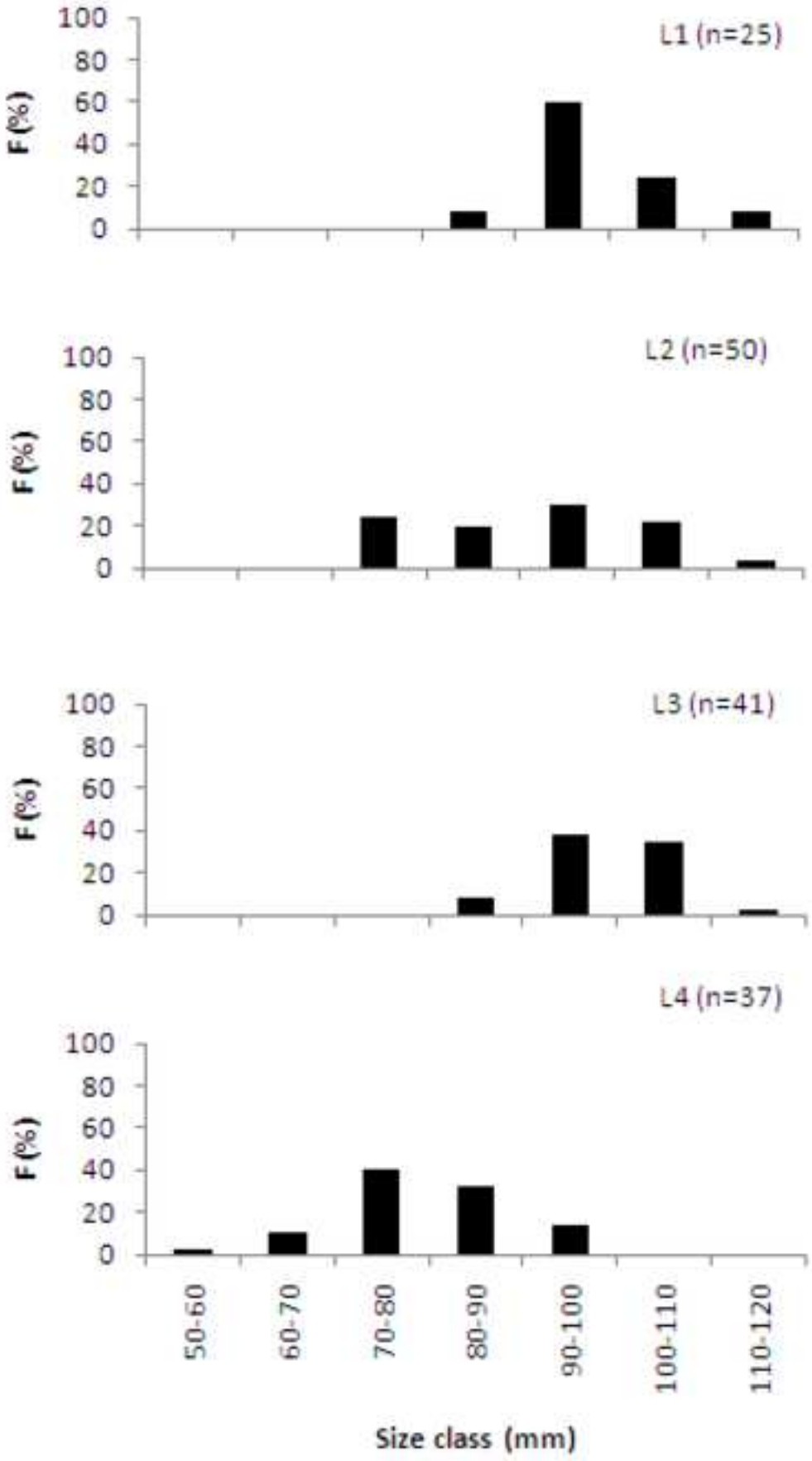

Figure 4

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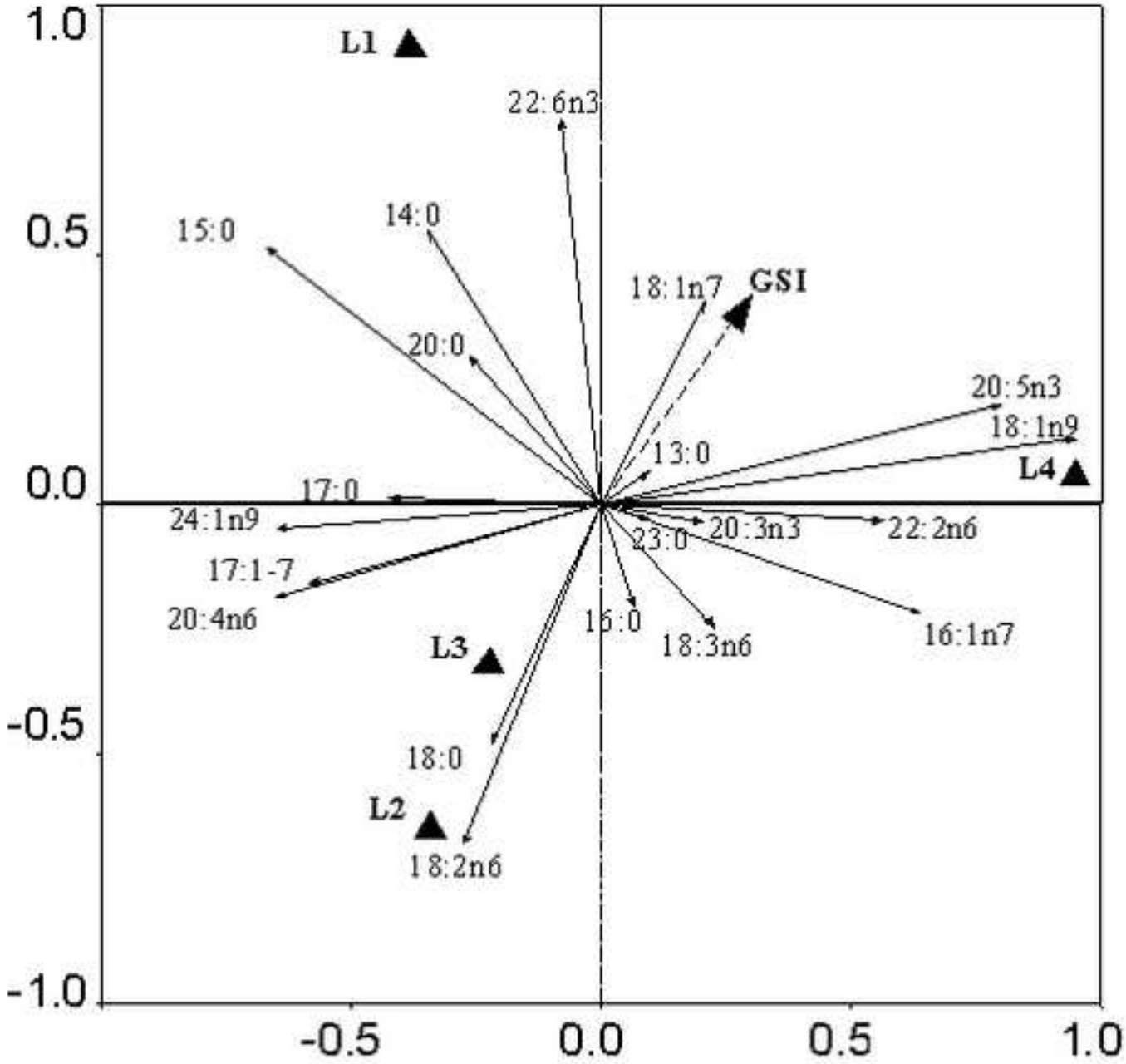
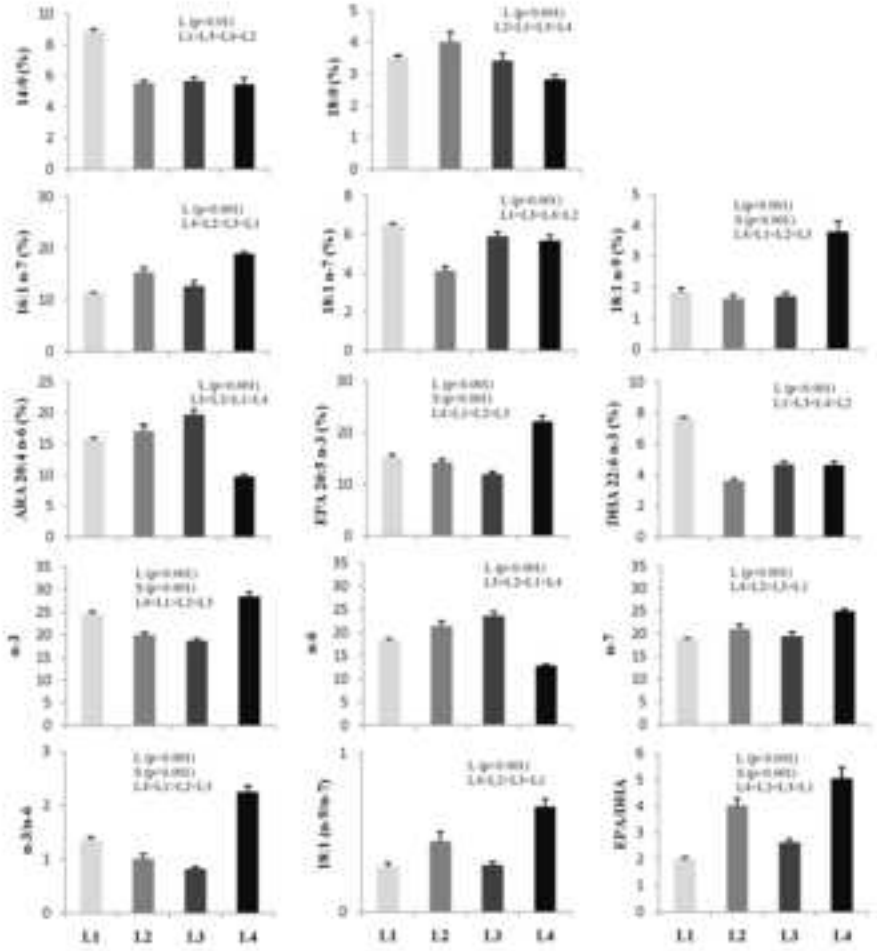


Figure 5



Research highlights

We analyzed the fatty acid (FA) composition of the gonads and potential food resources (mian algae and sediment samples) in order to assess the trophic relationships of this species. Three polyunsaturated FAs were the most abundant in the gonads of *S. purpureus*: 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

The FA composition of sea-urchin gonads changes in relation to sampling location (i.e. available resources) and the gonadal development.

Biomarkers of bacterial input and carnivorous feeding were more elevated in sea-urchins caught on bottoms with less vegetation

The results of this study highlight the omnivorous feeding behavior of this species and suggest that phytodetritus found within algae beds is an important carbon source for this species.