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► **To cite this version:**

C. Barberá, D. Fernández-Jover, J.A. López Jiménez, D. González Silvera, H. Hinz, et al.. Trophic ecology of the sea urchin elucidated from gonad fatty acids composition analysis. *Marine Environmental Research*, 2011, 71 (4), pp.235. 10.1016/j.marenvres.2011.01.008 . hal-00682416

HAL Id: hal-00682416

<https://hal.science/hal-00682416>

Submitted on 26 Mar 2012

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Accepted Manuscript

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

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PII: S0141-1136(11)00019-5

DOI: [10.1016/j.marenvres.2011.01.008](https://doi.org/10.1016/j.marenvres.2011.01.008)

Reference: MERE 3504

To appear in: *Marine Environmental Research*

Received Date: 12 November 2010

Revised Date: 18 January 2011

Accepted Date: 24 January 2011

Please cite this article as: Barberá, C., Fernández-Jover, D., Jiménez, J.A.López, Silvera, G., Hinz, H., Moranta, J. Trophic ecology of the sea urchin *Spatangus purpureus* elucidated from gonad fatty acids composition analysis, *Marine Environmental Research* (2011), doi: 10.1016/j.marenvres.2011.01.008

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

3

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14

15 **Abstract**

16

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

37

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

40 1. Introduction

41

42 Sea urchins are an important benthic megafaunal group, and play a significant ecologic
43 role in the community structure. Regular sea urchins are generally herbivores and feed
44 on micro- and macro-algae (Lawrence, 1975, 2007; Jangoux and Lawrence, 1982;
45 Verlaque, 1987; Carpenter, 1981), while urchins with a burrowing life trait are generally
46 considered detritivorous deposit feeders (Lawrence, 2007). Irregular urchins are
47 common in subtidal soft sediments around the world, and play an important role in the
48 biogenic disturbance (bioturbation) and biogeochemistry cycles in soft-sediment
49 systems (Chiold, 1989; Widdicombe and Austen, 1999; Widdicombe et al., 2004;
50 Lawrence, 2007; Lohrer et al., 2004, 2005, 2008). Large burrowing species such as
51 spatangoids (heart urchins) are particularly important for these processes due to their
52 abundance, size and mobility (Chiold, 1989). Large-scale losses of benthic bioturbators
53 due to fishing disturbances could impair the functioning of marine ecosystems (Thush
54 and Dayton, 2002). Despite the obvious ecological importance of burrowing urchins,
55 relatively little is known about the ecology of individual species, including their precise
56 dietary requirements (Lawrence, 2007; Jangoux and Lawrence, 1982). This is due to the
57 difficulties involved in traditional methods of stomach content analysis in which
58 ingested material is often unidentifiable due to digestion processes. Fatty acids have
59 recently been advocated as qualitative markers for tracing or confirming predator-prey
60 relationships in the marine environment (Grahnl-Nielsen et al., 2003; Iverson et al., 2004;
61 Budge et al. 2006), identifying key processes in the dynamics of pelagic ecosystems
62 (Brett and Müller-Navarra, 1997; Dalsgaard et al., 2003; Käkälä et al., 2005; Fernandez-
63 Jover et al., 2007), and examining trophic interactions within benthic ecosystems

64 (Ginger et al., 2000; Budge et al., 2002). The principle of this method is relatively
65 simple. Consumers derive their lipid requirements either from their diet or by
66 endogenous lipogenesis from dietary protein and carbohydrate precursors. Dietary lipids
67 are broken down into their constituent FAs and are incorporated relatively unchanged
68 into the tissues of the consumer (Lee et al., 1971; Stryer, 1995). Animals receive a
69 considerable amount of lipid via their diet, and thus the diet type alters the FA
70 composition of the organism (e.g.: Sargent and Whittle, 1981, Sargent et al., 1987;
71 Hughes et al. 2005; Hyne et al. 2009). Some polyunsaturated fatty acids (PUFAs) are
72 considered essential. These are FAs that are necessary but cannot be synthesized by the
73 organism, and thus must be consumed in the diet (Lenninger, 1986). Certain FAs, or
74 their ratios, have specific known sources and can therefore act as “trophic markers”,
75 providing a more precise indication of an organism’s diet than gut content analysis
76 (Sargent et al. 1987; Dalsgaard et al. 2003; Howell et al. 2003). In addition, the sea
77 urchins’ diet varies locally and depends on food availability (Vadas, 1977; Ayling,
78 1978; Beddingfield and McClintock, 1999). Diet quality has also been found to
79 influence somatic and gonadal growth and development in urchins (Emson and Moore,
80 1998; Cook et al., 2000; Liyana-Pathirana et al., 2002; Liu et al., 2007a, b), and
81 probably fecundity, as has been demonstrated in crustaceans (Hyne et al. 2009).

82

83 The spatangoid *Spatangus purpureus* (Müller, 1776) is widely distributed throughout
84 the Mediterranean and northwestern Atlantic (from the north of Africa to the north of
85 Europe and the Azores). This species has generally been described to be associated with
86 clean gravel or sandy substrata with low algal cover (Holme, 1966; Kanazawa, 1992);
87 however, around the Balearic Islands this species occurs in high abundances in

88 *Peyssonnelia* beds (between 30 m and 100 m depth) (Ordines and Massutí, 2009) where
89 its creates clearly visible furrows (Fig. 1). The present study aimed to elucidate the
90 trophic ecology of *Spatangus purpureus* on sandy bottoms of the Balearic Islands with
91 FA composition analysis, and had three main objectives: a) to elucidate the potential
92 food sources of *S. purpureus* (algae and/or sediment) by comparing the FA profiles; b)
93 to compare the FA profiles of the gonads from locations with different macroalgal
94 communities; and c) to analyze the changes in FA composition with respect to size and
95 gonad biomass, as changes in the diet are thought to affect individual growth and gonad
96 development.

97

98 **2. Materials and methods**

99

100 *2.1. Study area*

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

107

108 *2.2. Sampling method*

109

110 Samples for the FA composition analysis of gonads were collected over four days
111 (11/13/19/21 May 2009) during the MEDITS0509 survey (Data Collection Framework
112 for the Common Fisheries Policy) on board the R.V. Cornide de Saavedra. A 2-m beam
113 trawl was used to collect the sea urchins and algae. A box-corer was used to obtain
114 sediment samples. Four locations with sandy substrata but with different macroalgal
115 communities were selected (Fig.2): bare sand (L1) and algal beds on sandy bottoms
116 dominated by rhodoliths and other soft red algae such as *Peyssonnelia* spp.,
117 *Osmundaria volubilis* and *Phyllophora crispa* (L2, L3 and L4). Information on the
118 communities' distribution was based on a previous study (Ordines and Massutí 2009).
119 At each of the four locations three beam trawl samples were taken 100 m apart and the
120 sea urchin abundances were recorded. General information on the algal and faunal
121 composition was obtained from the same beam trawl samples (Table 1). The sampling
122 design incorporates two factors: location (L1, L2, L3 and L4) and site (each beam trawl
123 sample). A subsample of ten to fifteen individuals was taken at each location and site,
124 and the specimens were measured. The gonads of each individual were removed and
125 weighed. Five gonads of different individuals were randomly selected from each
126 location and site for FA analysis (between approximately 1 and 2 g of the gonad were
127 extracted) (4 locations x 3 sites x 5 replicates). Three subsamples of the three dominant
128 soft algae, *Osmundaria volubilis*, *Peyssonnelia* spp. and *Phyllophora crispa*, were also
129 obtained from the beam-trawl samples at three different sites of vegetated locations (5-6
130 g) (3 species x 3 sites x 3 replicates). At each location three grabs of sediment were
131 collected, from which two sediment subsamples were taken consisting of 5-6 g of
132 sediment from the first 4 cm of the surface (4 locations x 3 sites x 2 replicates). All
133 samples were frozen in glass tubes with Teflon-lined screw caps, and conserved at -
134 80°C 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 SPTM 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 140°C, and increased at a rate of 4°C
147 per min to 230°C, further increased at a rate of 1°C per min to 240°C and then held at
148 that temperature for 6 min. The injector and flame ionization detector were set a 250°C.
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/m²) 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/m²) (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

207 differences and was higher in L1 and lesser in L4 (Table 2). The size frequency
208 distribution pattern was different according to the location. In L1 sea urchins were
209 always larger than 80 cm, while in L4 there was a high proportion of smaller sea urchins
210 (Fig. 3). The GSI in the different locations was only compared for the size intervals 80-
211 90 cm and 90-100 cm because these were the most frequent in all four habitats. For size
212 class 80-90 cm, the GSI was highest in L1 and L4 and for size class 90-100 cm in L4
213 (Table 2).

214

215 3.3. Fatty acid composition of gonads, sediment and algae

216

217 Polyunsaturated fatty acids (PUFAs) dominated the lipids of the sea urchin gonads
218 ($41.95 \pm 0.77\%$), while saturated and monounsaturated fatty acids were present in
219 similar percentages (29.73 ± 0.59 and $28.31 \pm 0.60\%$ respectively). In algae, saturated
220 FAs dominated the lipid profile ($46.28 \pm 1.14\%$) followed by polyunsaturated FAs
221 ($37.83 \pm 1.37\%$). Monounsaturated lipids were the least abundant ($15.85 \pm 0.69\%$).
222 Sediments had high contents of saturated FAs ($47.08 \pm 3.62\%$), while monounsaturated
223 and polyunsaturated FAs were less abundant ($34.19 \pm 2.27\%$ and $18.72 \pm 2.82\%$,
224 respectively). Of the unsaturated FAs, the n-3 moiety predominated in *S. purpureus*
225 gonads ($22.93 \pm 0.66\%$), although the n-7 and n-6 fractions varied in second position
226 with similar proportions ($21.03 \pm 0.70\%$ and $19.02 \pm 0.72\%$ respectively). The n-6 FAs
227 ($20.68 \pm 1.32\%$) predominated in algae, followed by n-3 FAs ($17.16 \pm 1.61\%$). Finally,
228 n-7 and n-9 FAs were also abundant ($8.11 \pm 0.50\%$ and $7.72 \pm 0.45\%$ respectively). The
229 n-7 moiety ($18.67 \pm 2.12\%$) predominated in sediment, followed, with similar

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

232

233 The main FA components in the *S. purpureus* gonads were C20:4n-6 ($17.46 \pm 0.99\%$),
234 C16:0 ($16.32 \pm 0.22\%$), C20:5n-3 ($15.19 \pm 0.63\%$) and C16:1n-7 ($13.52 \pm 0.66\%$)
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 ($33.70 \pm 1.52\%$), C20:4 n-6
243 ($17.50 \pm 0.86\%$) and C20:5 n-3 ($9.00 \pm 0.86\%$) (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. crista* 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 ($26.74 \pm 0.69\%$), followed by C18:1n-9 ($14.40 \pm 1.12\%$) and C16:1 n-7 (13.42
250 $\pm 1.01\%$) (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

276 7.1% ($p=0.002$) of the explained variance of the model in the RDA analysis (Table 6,
277 Fig. 4). In the RDA graph GSI variable appears represented between locations with less
278 algal biomass (L1 and L4), locations where sea-urchins showed the more high
279 gonadosomatic index. The most representative FA for explaining this variance was
280 C18:1 n-7, which also showed significant differences in relation to location, and was
281 lower at L2 (Fig. 5).

282

283 In general, saturated and polyunsaturated FAs were similar at all the locations (Table 3),
284 and only the proportion of monounsaturated FAs was slightly higher at L4 (sand with
285 lower algal biomass) (ANOVA, $p<0.001$, S.N.K.: $L4>L3=L2>L1$) (Table 3). The n-3
286 moiety was significantly higher in the gonads of *S. purpureus* collected at L4 compared
287 with the other locations, followed by L1 (bare sand). There was a higher proportion of
288 the n-7 moiety in the gonads from L4. However, the n-6 moiety was less abundant in
289 this location; therefore, the ratio n3/n6 also showed significant differences in relation to
290 location (Fig. 5). The ratio C18:1 (n9/n7) was higher at L4 (bare sand) and L2
291 (rhodoliths and soft algae such as *Osmundaria volubilis*) and the proportion of
292 EPA/DHA was lower at L4 (Fig. 5).

293

294 **4. Discussion**

295

296 The current study shows that the FA profile of *S. purpureus* gonads can be a useful
297 trophic marker, as it was in good agreement with the FA composition of the potential
298 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).

323

324 Even though palmitic acid (C16:0) is an important component of the lipid fraction in the
325 gonads of *S. purpureus* and especially abundant in algae and sediment, it cannot be
326 considered an interesting trophic marker because it is present in high proportions in
327 many organisms (Řezanka and Sigler, 2009). However, this FA is less abundant in the
328 gonads of *S. purpureus* collected on bare sand bottoms, which could be related to low
329 algal availability. Palmitic acid can generate stearic acid (C18:0), which is used as a
330 precursor of monounsaturated FA by desaturation processes (formation of double
331 bonds). In animal tissues the most common monounsaturated FAs are palmitoleic acid
332 (C16:1n-7) and oleic acid (C18:1n-9), both precursors in the formation of polyunsaturated
333 FAs. Only the former FA was found in large proportions in both the sea urchin and
334 sediment samples, which suggests that it can be used as a trophic marker for mud
335 ingesters (Cook et al., 2000). Other FAs are also necessary for the formation of PUFAs,
336 such as linoleic acid (C18:2n-6) and alpha-linolenic acid (C18:3n-3), which belong to
337 the group of essential FAs (Lenninger, 1986). These FAs make up only a small part of
338 the composition of *S. purpureus* but they were used as diet markers for mud ingesters
339 and herbivorous organisms respectively (Table 7). However, this species may be able to
340 transform these essential FAs into the polyunsaturated FAs C20 and C22, which has
341 been demonstrated in other sea urchin species (Bell et al., 2001).

342

343 4.2. Origin of vegetal fatty acids

344

345 In general, PUFAs can be considered as trophic markers of a photosynthetic origin in
346 the diet (Cook et al., 2000; Brett and Müller-Navarra, 1997; Ikawa, 2004) and are

347 predominant components in the lipids of higher plants and animals. Eicosapentaenoic
348 acid (C20:5n-3) and arachidonic acid (C20:4n-6) were found in large proportions in the
349 gonads of sea urchins and the algae analyzed, and are representative components in the
350 FA profile of herbivorous organisms (Table 7). They are also essential FAs and serve as
351 precursors to eicosanoids, which are critical in a large range of physiological processes
352 (Lenninger, 1986). In general, they are present in considerable amounts in sea urchins
353 that feed on algae (Isay and Busarova, 1984; Cook et al., 2000; Hughes et al. 2005) and
354 some macroalgal species (Paradis and Ackman, 1977; Khotimchenko et al. 2002;
355 Nelson et al. 2002). Eicosapentaenoic acid (C20:5n-3) is characteristic of marine
356 invertebrates (Giddings and Hill, 1975) and is especially abundant in echinoderms
357 (holothurians) (Isay and Busarova, 1984; Romashina, 1983). In *S. purpureus* gonads it
358 is the third most important FA (11-22%), and there is a higher proportion in the gonads
359 of sea urchins from locations dominated by soft red algae (L4). Arachidonic acid, ARA
360 (C20:4n-6), is well represented in the gonads of *S. purpureus* from all the locations,
361 although its proportion is lower in the sea urchins collected at the locations with a low
362 algal biomass. Cook et al. (2000) found a predominance of this FA and stearidonic acid
363 (18:4 n-3) in the sea urchin *Psammechinus miliaris*, whose diet is mainly composed of
364 the alga *Laminaria saccharina*. High levels of EPA and ARA have also been found in
365 other macroalgal species (e.g. *Laminaria digitata*, *Alaria esculenta*) (Mai et al., 1996),
366 and it has been suggested that they are indicative of macroalgal material in the diet of
367 marine organisms (Sargent et al., 1987). Isay and Busarova (1984) found that high
368 levels of arachidonic acid are generally found in urchins and starfish, which could
369 indicate that these organisms have the ability to accumulate it in large quantities (Takagi
370 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

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

405

406 A large proportion of docosahexaenoic acid (C22:6 n-3) is related to a
407 carnivorous/necrophagus diet in benthic organisms (Pond et al., 1997; Cook et al., 2000,
408 2007; Gunstone et al., 1994). For example, myristic acid (C14:0) and docosahexaenoic
409 acid are significantly more abundant in the gonads of sea urchins collected on sand. In
410 this location, where there is little phytodetritus, sea urchins exploit the food that is
411 available and obtain the PUFA necessary for their metabolism by ingesting infauna or
412 animal detritus. Moreover, an increase in protein content in the diet can promote the
413 accumulation of docosahexaenoic acid and arachidonic acid as the gonadal tissue
414 matures (Cook et al., 2007). In fact, the GSI was higher in sea urchins collected on
415 sandy bottoms without algae or with low biomass. The sea urchins collected in the
416 present study were sampled at the same time of year and at similar depths to minimize
417 any temporal and bathymetric effects on the FA profiles (Budge et al., 2002; Lewis,
418 1967; Ferguson, 1976; Hughes et al. 2005). Therefore, differences in the gonadal index

419 and sizes could be explained by differences in growth or reproductive development due
420 to food availability and geographical differences (Harrold et al., 1985; Budge et al.,
421 2002).

422

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

438

439 4.4. Food quality and good fit at the different locations: the role of HUFA

440

441 It has been demonstrated that several aquatic organisms grow better when provided with
442 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

468 into the aquaculture of fish, molluscs and crustaceans has demonstrated that there are
469 strong dietary demands for HUFA-rich diets. The larval stages are more dependent than
470 adults because their high somatic growth rates cannot be satisfied by their FA
471 conversion capacities (Albentosa et al. 1994; Kanazawa and Koshio, 1994; Coutteau et
472 al., 1996; Sargent et al., 1999; Izquierdo et al., 2001).

473

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

484

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

486

487 The data on the high abundances of *S. purpureus* on soft bottoms with soft red algae in
488 the Balearic Islands (Ordines et al., 2009) can be considered evidence of the importance
489 of this habitat as a provider of detrital carbon. In this respect, *S. purpureus* plays a
490 potentially important role in biogeochemical processes and ecosystem functioning not
491 only on bare sand (as suggested by other authors) but even for deep sea algal
492 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 *Osmundaria volubilis* and *Phyllophora crispa* showed high levels of polyunsaturated

517 FAs, indicative of food quality. In the areas where these algae dominated the sea urchins
518 showed higher gonadal growth. This suggests that a mixed diet based on a higher
519 quality food source (both the sediment and algae) best explains the good fit of the sea
520 urchin populations, which has been demonstrated in other studies both in the laboratory
521 and the field (Cook et al. 1998, Fernandez and Bouderesque, 2000; Cook et al. 2000;
522 Hughes et al. 2005). The combined use of FA signatures, gonadal indices and growth
523 rates could be a useful tool for identifying the good fit of benthic species and identifying
524 essential habitats.

525

526 **Acknowledgements**

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

532

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802

803 **Figure legends**

804

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

808

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

811

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 \pm 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. Data on algal and faunal composition (mean \pm 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 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
L2	1585.14 \pm 316.26	1576.72 \pm 316.53	8.40 \pm 2.02	0.00 \pm 0.00
L3	8590.14 \pm 1866.50	1344.64 \pm 32.61	337.62 \pm 32.61	6752.49 \pm 652.16
L4	617.32 \pm 42.07	361.65 \pm 29.00	255.67 \pm 39.05	0.00 \pm 0.00
	Abundance fauna (ind/ha)	Biomass fauna (kg/ha)	<i>S. purpureus</i> (ind/ha)	
L1	4467.86 \pm 1797.37	49.03 \pm 17.90	54.41 \pm 32.81	
L2	4042.90 \pm 1151.84	134.53 \pm 6.49	445.80 \pm 148.50	
L3	200428.87 \pm 106193.27	922.40 \pm 398.11	696.00 \pm 264.00	
L4	42262.70 \pm 18956.57	518.57 \pm 414.35	2336.00 \pm 1877.00	

Table 2. Value of biological parameters (mean \pm 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 \pm 1.89	10.86 \pm 0.83	0.06 \pm 0.009	0.05 \pm 0.004
L2	50	92.66 \pm 1.72	6.34 \pm 0.76	0.02 \pm 0.008	0.03 \pm 0.002
L3	41	98.61 \pm 1.12	7.40 \pm 0.56	0.03 \pm 0.002	0.03 \pm 0.003
L4	37	79.49 \pm 1.60	6.64 \pm 0.85	0.05 \pm 0.006	0.07 \pm 0.003
ANOVA	p<0.001	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 <i>Spatangus purpureus</i>				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

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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 crispera*)

	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 (%).

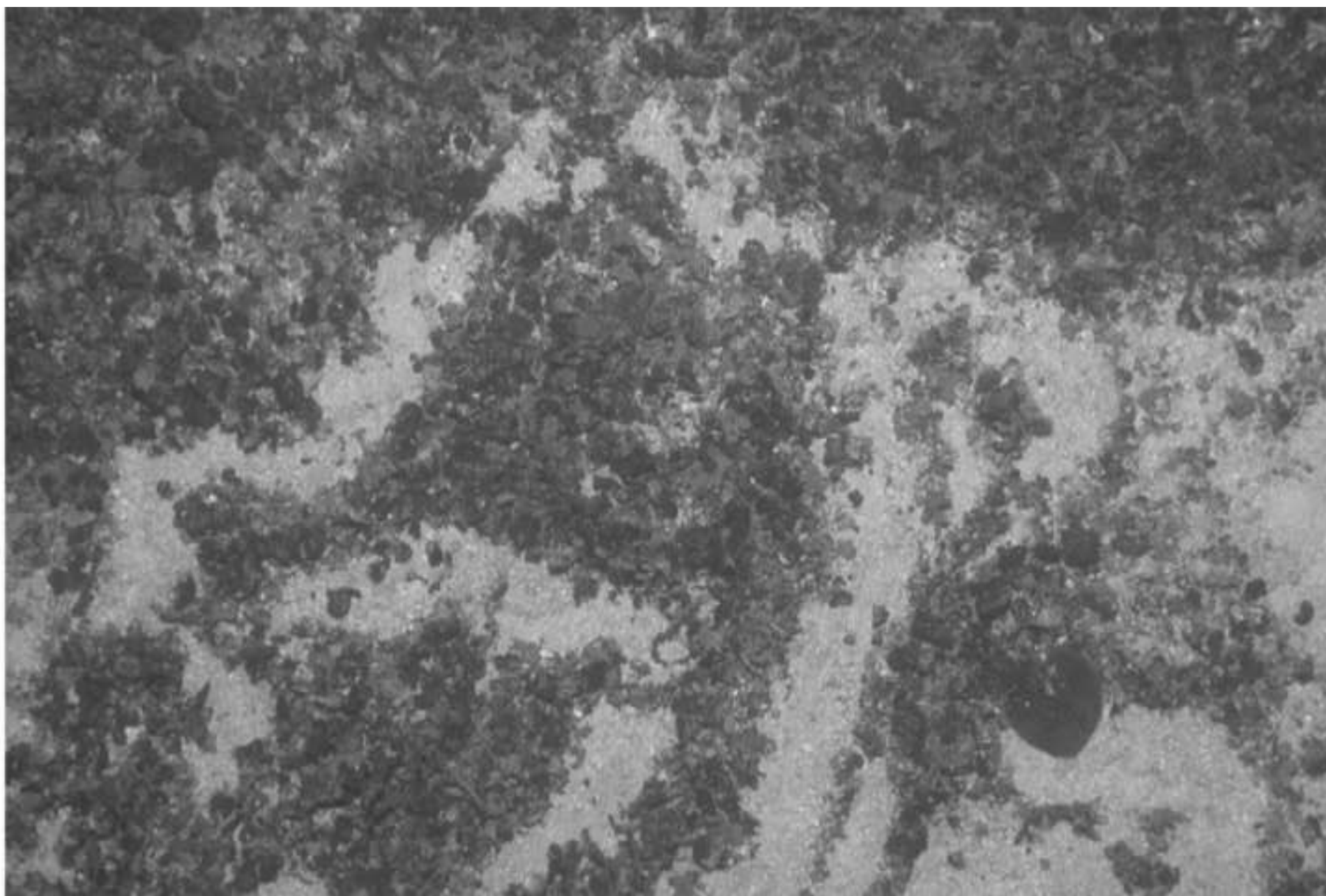
Similarity						Dissimilarity					
Gonads	All S=85.49%	L1 S=93.71%	L2 S=86.08%	L3 S=87.54%	L4 S=89.00%	Gonads	L1&L2 S=17.80%	L1&L3 S=15.57%	L1&L4 S=21.07%	L2&L4 S=20.31%	L3&L4 S=22.45%
C20:4 n-6	18.02	15.89	17.15	20.44	10.27	C16:1 n-7	13.01	11.2	17.96	9.89	13.94
C16:0	17.83	14.96	19.3	18.33	16.72	C22:6 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				

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. 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 Zooplankton	Howell et al., 2003; Sargent et al., 1987; 1995. Vashappilly and Chen, 1998; Mansour et al., 1999; Hughes et al. 2005 Kharlamenko et al., 2001
C20:5 n-3	Eicosapentaenoic (EPA)	Carnivorous feeding Diatomeas; Suspension feeding Macroalgal and sea urchins feeding on macroalgal	Tagaki et al., 1986; Kharlamenko et al., 2001; Cook et al., 2000; Budge et al., 2002; Hughes et al. 2005 Howell et al., 2003 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 Howell et al., 2003
C20:4 n-6	Arachidonic (ARA)	Protozoans, Microeucariotes in the sediment	Paradis and Ackman, 1977; Cook et al., 2000; Isay and Busarova, 1984; Mai et al., 1996 ; Nelson et al. 2002; Hughes et al. 2005 Howell et al., 2003
C18:2 n-6	Linoleic	Macroalgal and sea urchins feeding on macroalgal Mud ingesters	Paradis and Ackman, 1977; Cook et al., 2000; Isay and Busarova, 1984; Mai et al., 1996 ; Nelson et al. 2002; Hughes et al. 2005 Howell et al., 2003
C18:1 n-9	Oleic	Seagrass	Kharlamenko et al., 2001
C18:1 n-7	Vaccenic	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
C18:3 n-3	Alpha-linolenic	Seagrass	Kharlamenko et al., 2000; ;
C18:3 n-3	Alpha-linolenic	Algae	Nichols et al., 1982; Kayama et al., 1989; Nelson et al. 2002
C18:4 (n-3)	Stearidonic	Herbivorous Sea urchins feeding on algae	Sargent et al., 1983; Mai et al., 1996 Cook et al., 2000; Hughes et al. 2005
C18:4 (n-3)	Stearidonic	Algae	Kayama et al., 1989
C16:1 n-7	Palmitoleic	Bacteria; Mud ingesters ;	Cook et al, 2000 ; Gilian et al, 1988
C16:1 n-7	Palmitoleic	Diatoms	Ackman et al., 1968
C16:1 n-7	Palmitoleic	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



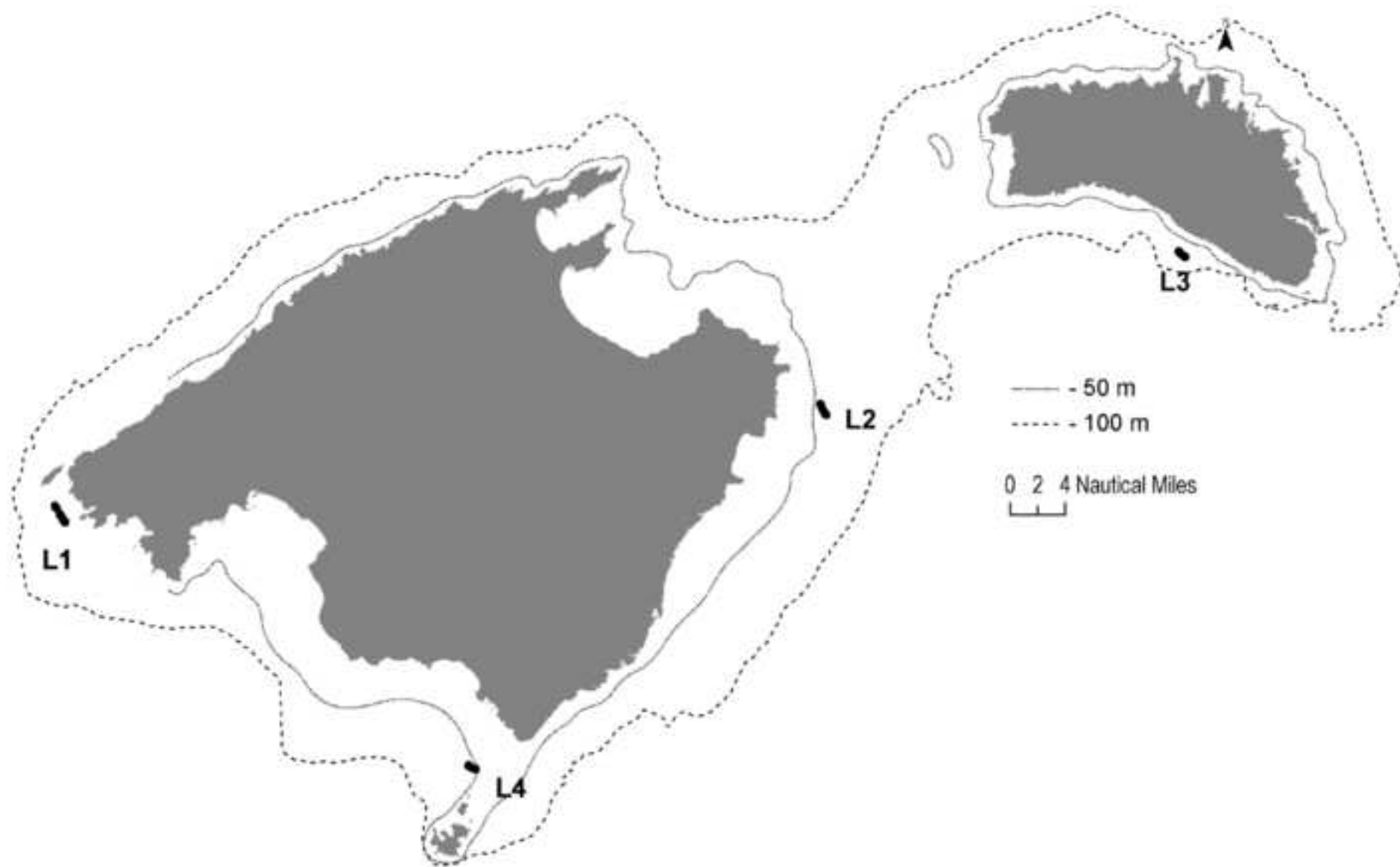


Figure 3

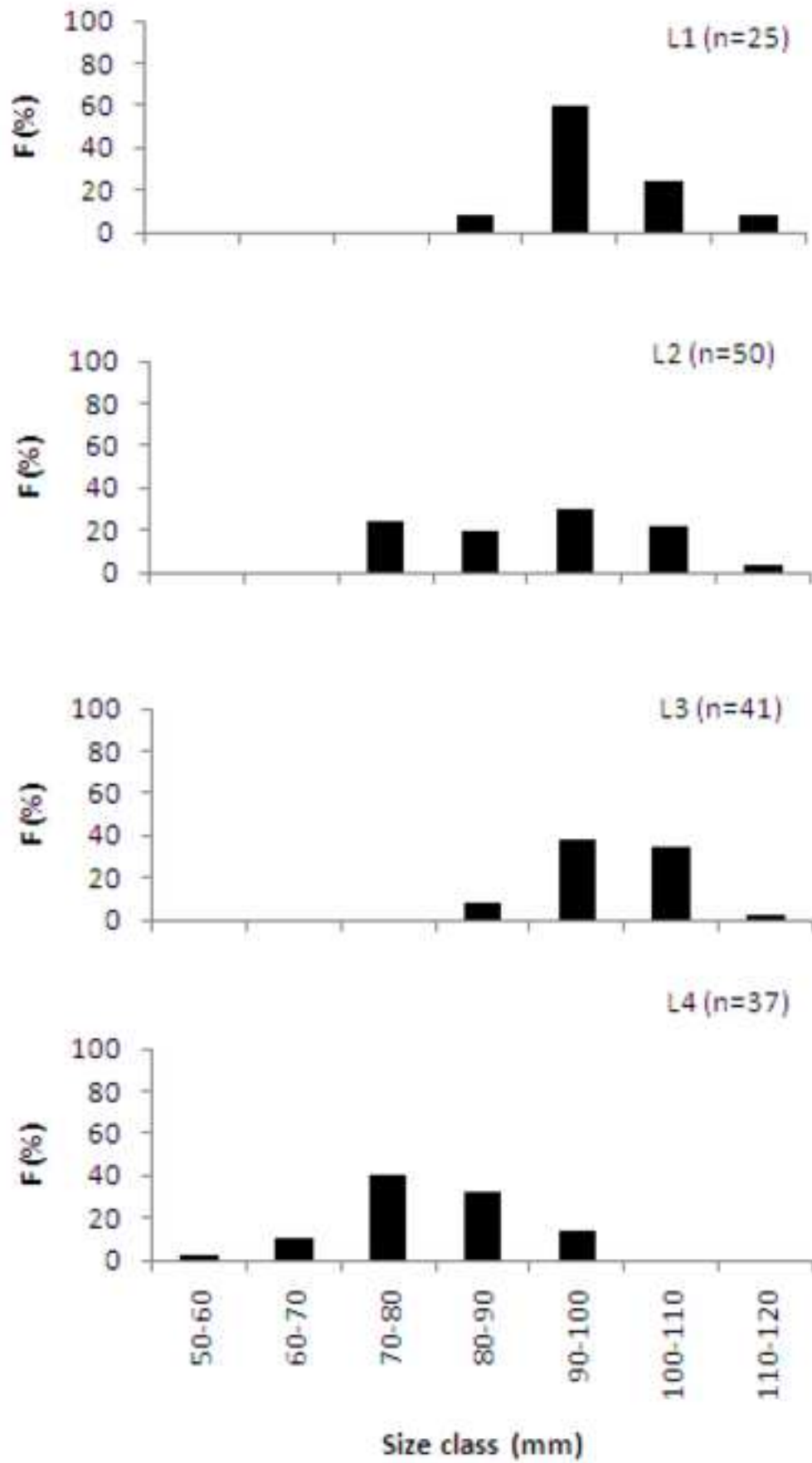


Figure 4

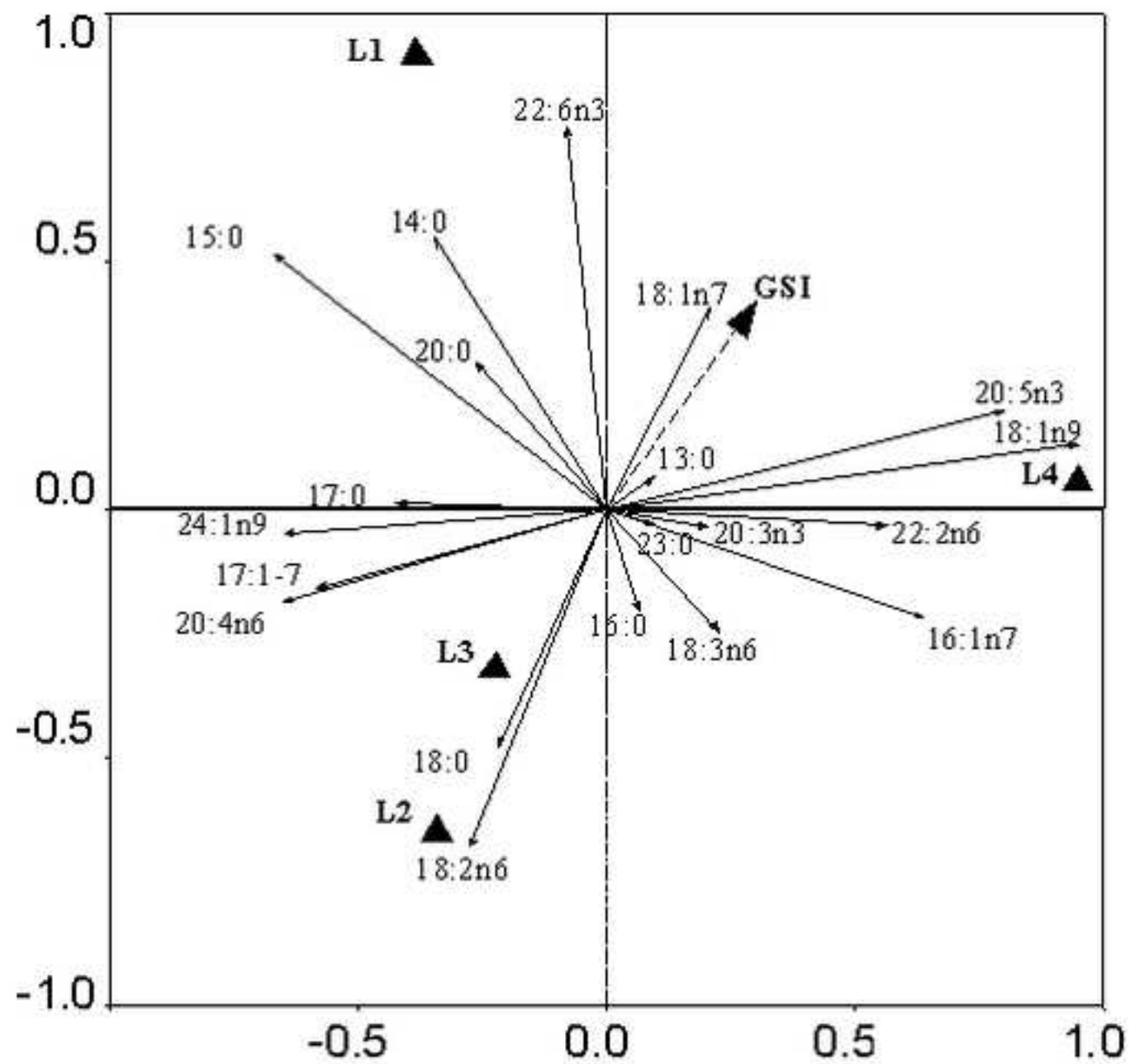
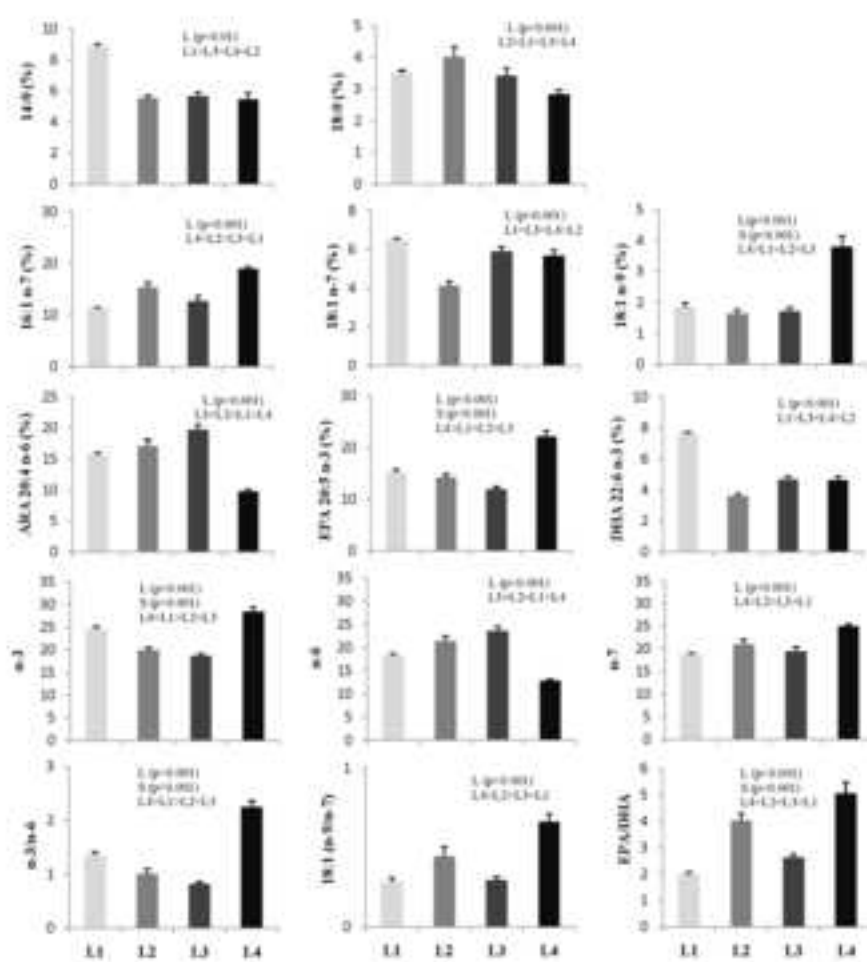
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Figure 5



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.