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3 from the Menez Gwen vent field

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22 ABSTRACT:

23

24 Specimens of the hydrothermal mussel *Bathymodiolus azoricus* collected in Menez Gwen
25 hydrothermal vent field (NE Atlantic) during 2002-2003 were examined for feeding patterns
26 variations through three seasons. The fatty acid profile and lipid classes of the mussels were
27 studied, together with the MODIS/AQUA derived near-surface chlorophyll *a* to test the hypothesis
28 that surface productivity might be related to the feeding patterns of this species. The lipid levels
29 showed pronounced seasonal fluctuations with the highest values occurring in January and August.
30 Seasonal variations in lipid classes and fatty acid composition of neutral and polar lipids in the
31 mussels are presented. Differences in the fatty acid profile of lipid classes in different seasons
32 suggest that the higher energy requirements in summer and winter were supplied by bacterial
33 biomarkers ω 7 MUFA (monounsaturated fatty acids), whereas ω 6 PUFA (polyunsaturated fatty
34 acids) and NMI (nonmethylene-interrupted) fatty acids predominated during the spring. The
35 MODIS/AQUA data show marked seasonal variability and an anomalous peak during January of
36 2003, although this cannot be directly linked to lipid composition variation.

37

38 KEY WORDS: Menez Gwen, *Bathymodiolus azoricus*; seasonality; lipids; fatty acids; biomarkers,
39 hydrothermal activity

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45 1. INTRODUCTION

46 All production in the deep-sea, with the exceptions of the hydrothermal vent environments is
47 fuelled, either directly or indirectly, by the import of organic matter to the bottom (Gage and Tyler,
48 1991), either as particulate matter (the detrital food chain) or by vertical migration (the grazing
49 food chain) (Raymont, 1983). Although primary production by chemosynthetic bacteria at
50 hydrothermal vents constitutes a rich source of organic carbon in the deep-sea, some vent species
51 do not meet all their nutritional requirements solely from this carbon source (Allen et al., 2001).
52 Seasonal variations in surface primary production may be reflected in vent species, particularly in
53 shallow vent fields such as the Menez Gwen (Mid-Atlantic Ridge, 840m), since surface particles
54 are estimated to sink at a rate of about 100 meters per day (Gage and Tyler, 2001). The mussel
55 *Bathymodiolus azoricus* dominates communities associated with deep-sea hydrothermal vents in
56 the Azores Triple Junction (ATJ) region, at depths ranging from 840 meters at Menez Gwen vent
57 field to 2300 meters at Rainbow vent field (Colaço et al., 1998; Desbruyères et al., 2001). This
58 species is known to live in dual symbiosis (with thio- or methanotrophic bacteria on its gills) (Fiala
59 et al. 2002; Duperron et al., 2006), and use the symbionts for nutritional purposes (Pond et al.,
60 1998; Colaço et al., 2002). The genus *Bathymodiolus* is considered a “generalist” in the
61 hydrothermal vent habitat, as it is able to take advantage of both suspension-feeding and the
62 production of its endosymbionts (Le Pennec et al., 1984). Some pelagic material has been found in
63 the gut of specimens (Colaço, 2001), which shows that this species is able to use the detritus food
64 chain.

65 Although primary production by chemosynthetic bacteria at hydrothermal vents constitutes a rich
66 source of organic carbon in the deep-sea, some vent species do not meet all their nutritional
67 requirements from this carbon source alone (Allen et al., 2001). The hypothesis that the particle
68 flux from the upper ocean layers can contribute to the carbon uptake by the mussel can be tested

69 by checking for biomarkers of surface primary producers in the mussel body, such as specific fatty
70 acids. In the Menez Gwen region, marked seasonal variation in near-surface Chl *a* and sea surface
71 temperature is evident from the results of this study. These Chl *a* cycles might be reflected in the
72 lipid composition of the vent mussel, especially in those from the shallowest vent field Menez
73 Gwen, since these are the biomarkers that persist in the pelagic food chain (Daalsgard et al, 2003).

74 Animal diets are usually verified by gut contents and faeces analysis or by behavioural studies.
75 However, these data only provide information on food consumption during a brief window of time,
76 and there may be a discrepancy between the diet ingested and the absorption / incorporation of the
77 different food items into the mussel tissues. Lipids are major sources of metabolic energy (neutral
78 lipids) and essential materials for the formation of cell and tissue membranes (polar lipids). The
79 fatty acid composition of marine organisms reflects to some extent the fatty acid pattern of their
80 food sources (Sargent et al., 1987; Howell et al., 2003) since the FA are destined either for
81 oxidation to provide energy (ATP) or for incorporation into phospholipids (Sargent, 1995; Sargent
82 et al, 1995). Diatoms, flagellates, macroalgae and bacteria may be distinguished by their fatty acid
83 composition. Fatty acids as dietary traces in the marine environment help to explore food origins
84 of various marine invertebrates (Howell et al., 2003; Suhr et al., 2003; Laureillard et al., 2004) and
85 the relationships in food webs within communities and marine ecosystems (Kharlamenko et al.,
86 1995; Phleger et al., 1999). For example, the fatty acids 18:4 ω 3; 20:3 ω 3; 22:5 ω 3; 22:6 ω 3 and
87 phytanic acid are photosynthetic biomarkers (Bergé and Barnathan, 2005). Therefore, comparison
88 of fatty acid profiles among mussels collected at different seasons can be used to gain further
89 information about the feeding habits of this bivalve. In order to acquire a better understanding of
90 the lipid composition of stored and structural fat of this species, this work was carried out during
91 three different seasons to i) investigate seasonal variations in lipid content and compositions, and
92 fatty acid compositions of the mussel *B. Azoricus* and ii) acquire a better understanding of the role
93 of photosynthesis-derived carbon on mussel nutrition.

94

95 2. MATERIALS AND METHODS

96 **2.1 Sampling**

97 During the SEAHMA (Seafloor and Sub-Seafloor Hydrothermal Modelling in the Azores Sea)
98 cruise in August 2002, specimens of deep-sea hydrothermal-vent mussels, *B. azoricus* Von Cosel,
99 Comtet and Krylova, were collected at the Menez Gwen vent field, located at approximately
100 37.51°N and 32.31°W (at 840 m depth), using the French ROV Victor 6000 and the French R/V
101 “L’Atalante”. At that time, retrievable cages (Dixon et al., 2001) were moored and filled with
102 mussels (~200 mussels per cage) using the ROV. These were then placed at diffuse venting areas
103 (the natural mussel habitat). These cages were then recovered in January and April 2003 with the
104 Portuguese R/V Arquipelago. Pruski and Dixon, (2003, 2007) showed that the retrievable cages
105 provided a much less stressful collection method than the ROV. The content of the mussel cages
106 was shared with other researchers for various studies. At the time of recovery, three mussel
107 samples from each recovery batch were dissected and soft tissue frozen at –80°C for subsequent
108 laboratory analyses.

109 **2.2 Lipid extraction**

110 Total lipids were extracted according to the Bligh and Dyer method (1959) as modified by White
111 et al., (1979). The lipids were then separated into different fractions using solid phase extraction
112 chromatography in silica gel columns (Isolute[®] SPE Columns). Non-polar lipid components were
113 separated with 7.5 ml of diethyl ether-hexane (1:1). The medium polar lipid fraction (mainly
114 glycolipids) was extracted with 7.5 ml of acetone and the high polar lipid fraction (mainly
115 phospholipids) was recovered with 15 ml of methanol. The absolute value of each lipid fraction
116 was obtained by weight after solvent evaporation. The relative percentage of lipid classes (polar

117 and non polar) and the different polar lipid components were determined by HPLC equipped with
118 an evaporative light scattering detector (ELSD) using the methodology previously used by
119 Bandarra et al. (2001).

120 **2.3 Fatty acid analyses**

121 Fatty acid methyl esters (FAMES) were prepared using base-catalysed transesterification with
122 sodium methoxide 0.5 M solution in anhydrous methanol (2 h at 30 °C), as proposed by Park et al
123 (2001) and Kramer et al (2002).

124 FAME analyses were performed in a Varian CP 3800 (Walnut Creek, CA, USA) gas
125 chromatograph equipped with an auto sampler and fitted with a flame ionisation detector at an
126 injection temperature of 250°C. The separation was achieved using a capillary column HP-
127 INNOWAX (30 m length, 0.25 mm internal diameter and 0.25 µm film thickness) from Agilent
128 (Albertville, MN, USA). Temperature was initially kept at 180°C for 5 minutes. Then, it was raised
129 at a rate of 4°C/minute up to 220°C, and maintained at 220°C for 25 minutes with the injector set
130 at 250°C. The split ratio was 100:1 and the measurement was taken using C21:0 as an internal
131 standard. The FAME identification was made by comparison to standards and whenever there was
132 any question about the results, an FA structural verification (see below) was performed. The fatty
133 acid profile was obtained by calculating the relative area percent of the chromatographic peaks
134 using C21:0 fatty acid as internal standard. All analytical determinations were made in triplicate.

135 The concentration of each FAME was reported as mg g⁻¹ dry weight of tissue.

136 **2.4 FA structural verification**

137 Due to the specific nature of the samples, which can have unusual fatty acids, a derivatization
138 method was used to further verify the mono and polyunsaturated double bond position of the
139 identified FAME. The 4,4-dimethyloxazoline (DMOX) (Fay and Richli. 1991) derivatives were

140 prepared by re-dissolving in 500 μl of 2-amino-2-methylpropanol (FLUKA) heating overnight at
141 180°C. After cooling, the reaction mixture was dissolved in 5 ml of dichloromethane and washed
142 twice with distilled water. The dichloromethane solution was dried with Na_2SO_4 and evaporated
143 under a stream of nitrogen flow at room temperature. The residue was dissolved in n-hexane for
144 analysis by gas chromatography–mass spectrometry (GC-MS).

145 The compounds were analysed with a gas chromatograph (Finnigan Trace gas chromatograph
146 ultra) coupled with a mass spectrometer (Finnigan Polaris Q mass spectrometer system - Thermo
147 Electron Corporation, MA, USA)). A splitless injection was performed with 1 μl of sample. The
148 carrier gas was helium at 10 Psi. A 25 m x 0.25-mm id x 0.25 μm HP-5[®] (Hewlett-Packard)
149 column was used. The GC-MS conditions for DMOX derivatives elution were as follows: started
150 with 2 minutes at 90°C, followed by a 5°C min^{-1} ramp up to 280°C over 20 minutes.

151 **2.5 Chlorophyll *a* concentration and surface temperature measurements derived from** 152 **satellite images**

153 Monthly MODIS/AQUA-derived near-surface chlorophyll *a* (Chl *a*) and sea surface temperature
154 (SST) images were obtained for a region above the mid-Atlantic Ridge at approximately 37°N,
155 31°W (Menez Gwen). MODIS spatial resolution is 1.1 km resolution. Chlorophyll *a* and SST
156 images were obtained using the Ocean Chlorophyll 3 bands OC3M (O'Reilly et al. 2000) and
157 long-wave SST (LW-SST) (Franz, 2006) algorithms, respectively. Regular daily MODIS images
158 were obtained from the Ocean Colour Level 1/2 browser (OceanColor Web 2006). These images
159 are mapped (Level2-map) with SeaDAS, and a master file was created specifically for the Menez
160 Gwen region. The download and mapping process is automated within the HTRP (High Resolution
161 Picture Transmission) station in the Azores HAZO- system developed by Figueiredo et al. (2004).

162 MODIS data was used to study the monthly and inter-annual surface pigment and temperature
163 variability at Menez Gwen between 2002 and 2008. Only Chl *a* values below 0.05 and above 7 mg

164 m^{-3} were excluded from further analysis due to pixel contamination from clouds, aerosols and/or
165 suspended sediment. Only SST values above 10°C and below 30°C were used to avoid “false” low
166 water temperature values due to pixel cloud contamination.

167

168 **2.6 Statistical analysis**

169 The Kruskal Wallis test (Zar, 1999) was applied to test for seasonal differences in polar and
170 neutral lipid composition of *B. azoricus*. A significance level was established at 0.05.

171 A principal component analysis (PCA) (Legendre and Legendre, 1998) was performed using the
172 different FA profiles as descriptors for different individuals in order to determine whether the polar
173 lipids and neutral lipids differ in specific seasons and between seasons.

174

175 **3. RESULTS**

176

177 **3.1 Lipid classes**

178 The total lipid content of *B. azoricus* varied significantly (Kruskal-Wallis test: $H(3, 3, 3) = 7.20$ p
179 $= 0.027$) with the season (Table 1). The lowest levels were found in spring, while in summer the
180 mussels showed approximately a three-fold increase. The relative proportion of polar and non-
181 polar lipid content in the total tissue also showed a tendency to vary throughout the year.

182 The non polar lipid content as a percentage of total lipids also showed seasonal variation (see
183 Table 1). The highest levels were found in winter (91%), while spring and summer showed the
184 same percentages (76%).

185 The polar lipid fraction was lowest during winter (8.5%), with the spring and summer showing
186 similar percentages (%).

187 Phosphatidylethanolamine (PE) and cardiolipine (CL) were most abundant during summer, while
188 the phosphatidylcholine (PC) reached its peak level in spring.

189 **3.2 Fatty acids**

190 The fatty acid profile of polar lipids (PL) and non-polar lipids (NPL) was analyzed for the winter,
191 spring and summer months. A wide variety of fatty acids were detected (Table 2).

192 The most common fatty acid for both lipid classes was the C16:0 and 16:1 ω 7.

193 In the PL fraction, 20:3 ω 6 showed the highest percentage, reaching over 50% during winter. The
194 second most common fatty acid was 16:1 ω 7, which showed higher levels in summer than in
195 winter. The fatty acids 18:2 ω 6 and 20:1 ω 7 showed higher percentages in spring and summer.
196 Nevertheless, in these seasons 22:6 ω 3 appeared in significant amounts. Although the C18:1 group
197 represented approximately 6 % of the polar lipids in all seasons, the highest contributor to the
198 MUFA in all lipid classes in the spring and summer was 16:1 ω 7. The highest percentages of
199 PUFA were present during winter and the lowest in the spring and summer. Branched fatty acids
200 showed no evidence of seasonal variation.

201 Levels of fatty acids 18:4 ω 3 and C20:4 ω 6 were higher in the polar than in the non polar fraction.

202 The most common fatty acid in non polar lipids was the 16:1 ω 7. Levels of this fatty acid decrease
203 in spring and increase in summer. Fatty acids 20:1 ω 7 and 22:2 ω (9,15) were also important
204 components (>5%) in this lipid fraction in all seasons. However, it was observed that the registered
205 levels of 22:2 ω (9,15) were much higher in spring (>20%) than during other seasons
206 (approximately 5%).

207 In non-polar lipids MUFA showed a significant decrease during spring. This decrease was mainly
208 due to the ω 7 fatty acids. PUFA showed highest levels in spring. Despite the low quantities,
209 branched fatty acids were more important in winter compared with other seasons. The NMI fatty

210 acids were more abundant in NPLs and reached their peak levels in spring. The non methylene
211 interrupted dienoic (NMID) fatty acids C20:2 was very important to the non-polar fraction in all
212 seasons, with C22:2 being very important in the spring.

213 The polar lipid fraction also showed a high proportion of C20:3 ω 6, while in the non-polar fraction,
214 C20:1 ω 7 and the NMI C22:2 ω (9,15) represented an important part of the total lipids.

215

216 Photosynthetic biomarkers did not appear in significant percentages in the samples analysed. There
217 were no significant differences between seasons and the highest percentage found was in the PL
218 fraction. Despite the lack of seasonal variation in the ω 3 fatty acids family of both lipid fractions,
219 the percentage of ω 3 and ω 6 PUFA in the PLs was significantly higher ($p < 0.05$) than in the NPL,
220 with the exception of ω 6 fatty acids, whose levels increased during the spring.

221 The PCA showed that there were no major differences between the different lipid classes and
222 seasons. The first component explains 71.73% of the variance and all the individuals are grouped
223 in one end of the first component. The second component explains only 16.85% of the variance
224 The individuals from the winter sampling showed more differences, mainly due to fatty acid
225 C20:3 ω 6, which contributed by almost 67% to the second factor (Fig.2).

226 3.4 Satellite Data

227 MODIS near-surface Chl *a* (mg m^{-3}) and surface temperature (SST in $^{\circ}\text{C}$) monthly medians were
228 calculated for the Menez Gwen region from June 2002 through to March 2008 (Fig. 1). During this
229 period of time, strong spring Chl *a* bloom signals were evident. Spring blooms began at different
230 times in different years. During the first two years (2002 and 2003), late spring blooms
231 (April/May) were observed, while in the later years the blooms commenced earlier (March/April).
232 The highest Chl *a* monthly averages ($0.30 - 0.35 \text{ mg m}^{-3}$) were found during the years 2003, 2005,
233 and 2008 and the lower averages ($0.05 - 0.09 \text{ mg m}^{-3}$) were found in 2002, 2004, 2006 and 2007.

234 In addition, Chl-*a* autumn blooms of a smaller amplitude were more or less visible in 2002-2003
235 while late “autumn” blooms (Dec/Jan) were observed in subsequent years (2004 to 2007). For all
236 years, the lowest monthly Chl *a* averages were observed during summertime.

237

238 4. DISCUSSION

239 The total lipid content of *B. azoricus* varied markedly ($p < 0.05$) depending on the season. The
240 lowest values were found in spring, while in summer the mussels showed an approximately three-
241 fold increase. Since phytoplankton blooms occur in the spring at this latitude in surface waters (see
242 Fig. 1), this result was somewhat unexpected. Nevertheless, if they feed primarily on material
243 resulting from zoo- and phyto-degradation, there would be a time lag, which would tend to explain
244 the higher levels in summer.

245 Winter was the period with the lowest level of PLs in membranes, probably due to the utilization
246 of some structural lipids to maintain the body during periods of food scarcity and reproduction.

247 The high NPL content in the winter suggests that a significant portion of the total lipids exist as
248 energy reserves in this season. The relative amounts of these compounds, however, varied along
249 the year. According to Colaço et al (2006) and Dixon et al (2006), this species is sexually mature
250 in winter. The gonadal cell proliferation must at some stage involve intense biochemical synthesis
251 of protein and lipid reserves for egg production, thus explaining the high level of NPLs (storage
252 lipids) during winter (Holland, 1978). Since sexual reproduction is an energy-intense process, the
253 dramatic decrease in NPLs after winter suggests that the this lipid class may be used as energy
254 reserves for reproduction purposes, and the subsequent decrease observed is probably correlated
255 with spawning. Several authors (Gardner & Riley 1972; Swift, 1977; Beninger & Stephan, 1985,
256 Pazos et al. 1997 and Soudant et al. 1999) observed changes in the neutral lipids in other mytilids

257 due to reproduction, whereas the polar lipids were influenced to a lesser extent and remained
258 practically constant over the year.

259 The higher PE and CL values in summer are in accordance with the highest levels of bacterial fatty
260 acid biomarkers present in this season, since PE is frequently the main lipid component of
261 microbial membranes, and CL is found only in the membranes of bacteria and mitochondria
262 (Kraffe et al, 2005). The PC is usually the most abundant phospholipid in animal and plant tissue
263 membranes, often amounting to almost 50% of the total (Christie, 1989). On the other hand, it is
264 less often found in bacterial membranes, probably in only 10% of species (Christie W.L. 2007). Its
265 higher level in spring may be related to the abundant availability of particles due to the spring
266 bloom. The low level of PC in winter, the period with the lowest level of structural lipids, confirms
267 the probable use of this phospholipid in mussel lipid body metabolism maintenance.

268

269 **4.1 Fatty acid composition:**

270 The fatty acid composition of an animal can be used as an indicator of its trophic ecology (Sargent
271 et al. 1987, Colaço, et al. 2007). Deep-sea hydrothermal vent animals use the organic metabolites
272 produced by bacteria as a food source (Pond et al. 2000; Colaço et al. 2002), and until recently it
273 was generally accepted that the contribution of photosynthetic-derived material was negligible.

274 In the present work, the presence of the ω 7 fatty acid family and the NMID, whether in the NPL or
275 PL fraction, indicate a clear dependence on bacterial-derived carbon in all seasons. The presence
276 of abundant NMID C22:2 ω (9,15) in NPLs but not in PLs could indicate an inability to use this
277 fatty acid for physiological or structural purposes and thus might explain its accumulation in NPLs
278 for energy needs as observed by Sato and Ando (2002) in brittle stars.

279 The presence of phytanic acid, even in small proportions, reflects utilization by the vent mussels of
280 organic material created in the photic zone during the photosynthesis processes. However the ω 3

281 family of fatty acids did not show seasonal variations like those of the fatty acids from the $\omega 6$
282 family. The former appeared in higher percentages in the NPL fraction in spring, while in the PL
283 fraction the higher percentages were found in winter, mainly due to the fatty acid 20:3 $\omega 6$. The
284 presence of greater quantities of the $\omega 3$ family in the PL fraction than in the NPL fraction seems to
285 indicate that the former are principally incorporated and used for structural functions, rather than
286 for energy purposes, which is congruent with their role as essential fatty acids. In marine animals
287 the fatty acids comprising the lipid reserves are mainly saturated or monounsaturated (Pond et al.
288 2000), which is in line with the results of this work.

289 In PLs, the high level of PUFA 20:4 $\omega 6$ measured during wintertime may be related to intense
290 seasonal biochemical synthesis of reproduction lipid structures, which takes place during this
291 season. This fatty acid is a precursor of prostaglandins, and prostaglandins play a key role in the
292 reproductive processes of molluscs (Morse 1984).

293 In this study, branched fatty acids did not change during the year, but the results suggest that they
294 may contribute towards an implementation of reserve lipids during winter, as reflected in the
295 greater amounts of non-polar lipids found during this season.

296 The composition of the fatty acid profile of *B. azoricus* confirms the existence of a food chain
297 based on bacterial chemosynthetic rather than phytoplanktonic primary production (Fisher 1990).
298 However, the presence of trace amounts of phytanic, 22:5 $\omega 3$ and 22:6 $\omega 3$, which are of
299 phytoplanktonic origin, is probably related to the ability of the vent mussel to filter-feed on
300 external particulate material.

301 The hydrothermal mussels have ingested this available material. The slow uptake of fatty acids in
302 the PL class explains the high levels of total lipids in the summer. This fact could explain the
303 higher amounts of PUFAs $\omega 3$ and other phytoplankton biomarkers such as $\omega 6$ in NPLs during
304 spring.

305 5. CONCLUSION

306 The presence of phytanic acid reflects the utilization of surface organic material by the vent
307 mussels. However, phytanic acid and other photosynthetic-derived fatty acids are never present in
308 large quantities in this species, even when phytoplankton blooms are evident in surface waters.
309 This species relies mainly on chemosynthetic-derived material, and the variations observed are
310 probably due more to physiological stages (e.g. reproduction) than to seasonal variations of sea
311 surface water productivity.

312 Further studies will be fundamental to improving our understanding of how this surface-bottom
313 relationship is established / maintained and to quantify its importance.

314

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327

328 7. REFERENCES

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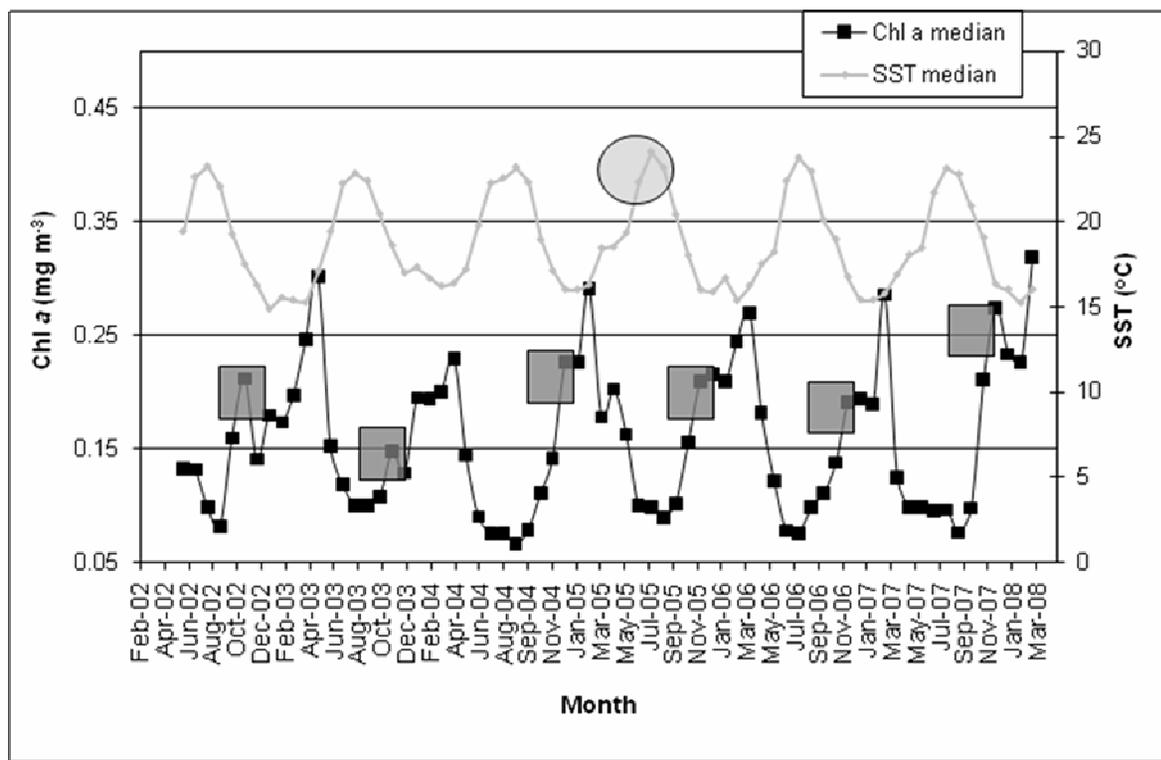
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Fig. 1. MODIS/AQUA monthly median SST (in °C) and Chl *a* (in mg m⁻³) values for the Menez Gwen region from 2002-2008. Large dark grey squares represent autumn Chl *a* blooms, while the light grey circle represents the highest monthly median temperature value for the region.

Fig. 2. Principal component analyses with individuals as cases and fatty acids as descriptors. Winter stands for individuals collected in Winter; Spring for individuals collected in spring and Summer for individuals collected in Summer. N stands for non-polar and P stands for polar fatty acids. Component 1 represents 71,73% of the variance, while the component 2 represents 16,85% of the variance.

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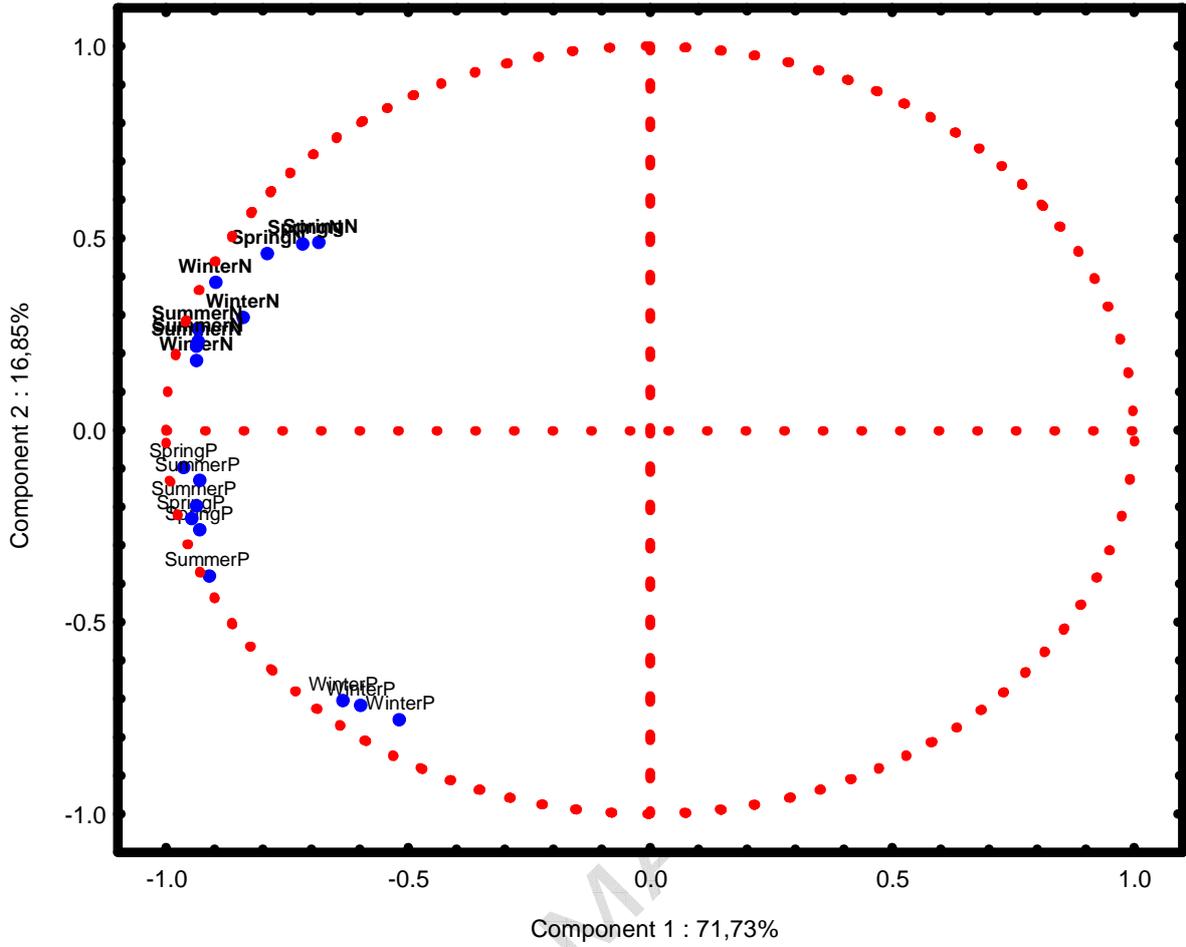


Fig.2

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Table 1. Lipid class composition by season of the mussel *Bathymodiolus azoricus*.

	Total lipids (mg g ⁻¹ dw)	Non-polar lipids (%)	Polar lipids (%)				
			PE	CL	PC	X	Total %
Winter n=2	5.7 ± 1.2	91.3	6.8	0.3	1.2	n.d.	8.4
Spring n=3	3.3 ± 0.7	76.1	7.3	n.d.	14.0	1.9	23.2
Summer n=3	9.9 ± 2.6	76.4	14.5	3.3	4.5	1.0	23.3

PE - Phosphatidylethanolamine; CL - Cardiolipine; PC - Phosphatidylcholine; X - Unknown Molecule
n.d.-not detected

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Table 2. Fatty acid composition (mean percentage of total fatty acids \pm SD) of non-polar and polar lipids and total lipids extracted from mussels *Bathymodiolus azoricus* collected from the Menez Gwen vent field in different seasons. Total lipids are given as

mg g^{-1} dw (mean \pm SD).

Fatty acids	Winter		Spring		Summer	
	Non-polar lipids	Polar lipids	Non-polar lipids	Polar lipids	Non-polar lipids	Polar lipids
12:0	0.03 \pm 0.06	0.1 \pm 0.08	0.04 \pm 0.03	0.18 \pm 0.05	nd	0.05 \pm 0.02
13:0	0.08 \pm 0.09	0.17 \pm 0.15	0.03 \pm 0.01	0.19 \pm 0.03	0.03 \pm 0.01	0.03 \pm 0.01
14:0	0.01 \pm 0.02	0.94 \pm 0.86	0.01 \pm 0.01	1.92 \pm 0.60	0.01 \pm 0.01	2.22 \pm 0.52
14:0 isobr.	0.05 \pm 0.02	0.11 \pm 0.09	0.03 \pm 0.00	0.15 \pm 0.10	0.04 \pm 0.02	0.19 \pm 0.02
15:0	0.10 \pm 0.05	0.2 \pm 0.18	0.10 \pm 0.01	0.31 \pm 0.06	0.07 \pm 0.01	0.23 \pm 0.01
15:1	0.06 \pm 0.05	0.14 \pm 0.13	0.06 \pm 0.04	0.11 \pm 0.06	0.03 \pm 0.01	0.06 \pm 0.04
16:0	8.09 \pm 4.27	6.05 \pm 5.20	5.15 \pm 1.17	10.03 \pm 0.85	8.09 \pm 0.67	9.90 \pm 0.315
16:1 ω 9	0.52 \pm 0.89	0.11 \pm 0.10	0.63 \pm 0.59	0.91 \pm 0.17	nd	0.43 \pm 0.14
16:1 ω 7+ ω 6	28.5 \pm 9.76	5.30 \pm 5.22	13.61 \pm 2.10	19.38 \pm 1.57	34.52 \pm 3.36	27.24 \pm 1.28
16:0 iso	0.17 \pm 0.12	0.05 \pm 0.04	0.14 \pm 0.10	0.07 \pm 0.01	0.05 \pm 0.02	0.07 \pm 0.03
16:0 anteiso	5.91 \pm 8.56	0.73 \pm 0.63	0.76 \pm 0.23	0.90 \pm 0.19	1.70 \pm 2.50	0.20 \pm 0.06
Phytanic	0.01 \pm 0.01	0.09 \pm 0.12	0.01 \pm 0.01	0.13 \pm 0.03	nd	0.07 \pm 0.04
17:0	0.04 \pm 0.01	0.14 \pm 0.19	0.04 \pm 0.01	0.50 \pm 0.11	0.03 \pm 0.01	0.29 \pm 0.04
17:1 ω 6+ ω 7	0.11 \pm 0.06	0.06 \pm 0.09	0.18 \pm 0.01	0.22 \pm 0.17	0.17 \pm 0.02	0.14 \pm 0.01
16:4 ω 3	0.20 \pm 0.09	1.32 \pm 1.07	0.41 \pm 0.13	0.93 \pm 0.14	0.12 \pm 0.05	1.52 \pm 0.04
18:0	0.03 \pm 0.00	1.8 \pm 1.59	0.03 \pm 0.03	3.14 \pm 0.61	0.04 \pm 0.07	2.23 \pm 0.24
18:1 ω 11	0.02 \pm 0.02	0.08 \pm 0.07	0.01 \pm 0.02	0.87 \pm 0.18	nd	1.10 \pm 0.25
18:1 ω 9-c* ω 13	1.25 \pm 0.39	4.92 \pm 1.36	1.46 \pm 0.34	3.80 \pm 1.41	1.73 \pm 0.10	2.55 \pm 0.46
18:1 ω 7+ ω 6+ ω 8	0.43 \pm 0.23	1.74 \pm 0.57	0.45 \pm 0.10	4.24 \pm 1.15	0.67 \pm 0.10	3.49 \pm 0.27
18:3 NMI	0.54 \pm 0.22	1.36 \pm 0.28	0.47 \pm 0.07	5.56 \pm 1.64	0.87 \pm 0.17	8.07 \pm 0.73
18:3 NMI	0.07 \pm 0.03	0.05 \pm 0.05	0.06 \pm 0.02	0.12 \pm 0.05	4.06 \pm 0.21	0.03 \pm 0.01
18:2 ω 6	0.60 \pm 0.11	0.36 \pm 0.10	0.04 \pm 0.06	0.80 \pm 0.39	1.34 \pm 0.09	0.78 \pm 0.08
18:4 ω 3	0.16 \pm 0.13	1.66 \pm 0.88	0.45 \pm 0.33	2.31 \pm 0.46	0.24 \pm 0.01	3.00 \pm 0.47
20:0	0.16 \pm 0.05	0.07 \pm 0.13	0.53 \pm 0.44	0.19 \pm 0.09	0.15 \pm 0.03	0.91 \pm 1.48
20:1 ω 8+ ω 14	0.23 \pm 0.06	0.63 \pm 0.10	nd	3.67 \pm 0.92	0.07 \pm 0.06	3.79 \pm 0.49
20:1 ω 7+ ω 6	6.73 \pm 2.39	0.78 \pm 0.03	5.78 \pm 0.97	4.19 \pm 2.18	9.82 \pm 0.51	5.33 \pm 0.89
20:2 ω 6	0.15 \pm 0.08	0.19 \pm 0.10	0.12 \pm 0.05	0.87 \pm 0.21	0.23 \pm 0.05	1.14 \pm 0.13
20:3 ω 6	0.02 \pm 0.03	50.58 \pm 20.29	0.01 \pm 0.01	11.02 \pm 2.60	nd	10.87 \pm 1.38
20:2 ω (9,15)+ ω (7,15)+ ω (6,15)	3.43 \pm 2.93	nd	2.63 \pm 0.62	0.06 \pm 0.07	0.92 \pm 0.04	0.07 \pm 0.09
20:3 ω 3	0.08 \pm 0.06	0.16 \pm 0.15	0.01 \pm 0.01	0.27 \pm 0.04	0.09 \pm 0.05	0.17 \pm 0.04
20:3 ω 6	0.03 \pm 0.02	0.1 \pm 0.09	0.09 \pm 0.03	0.11 \pm 0.01	0.07 \pm 0.02	0.06 \pm 0.03
20:4 ω 6	0.02 \pm 0.02	2.22 \pm 1.02	0.74 \pm 0.62	3.12 \pm 2.16	0.04 \pm 0.02	0.59 \pm 0.47
22:0	0.07 \pm 0.12	nd	nd	0.26 \pm 0.07	0.01 \pm 0.01	0.04 \pm 0.05
22:1 ω 11	nd	0.11 \pm 0.12	nd	0.78 \pm 0.06	nd	0.66 \pm 0.26
22:1 ω 9	0.71 \pm 0.67	0.38 \pm 0.11	0.21 \pm 0.03	0.47 \pm 0.08	0.32 \pm 0.18	0.26 \pm 0.31
22:1 ω 7	2.43 \pm 0.92	1.82 \pm 0.87	7.98 \pm 0.10	3.82 \pm 1.02	0.96 \pm 0.17	2.81 \pm 0.53
22:3 NMI	1.78 \pm 0.92	0.06 \pm 0.09	2.01 \pm 0.30	0.60 \pm 0.24	1.96 \pm 0.07	0.34 \pm 0.03
22:2 ω (9,15)	6.36 \pm 3.54	0.04 \pm 0.06	20.67 \pm 2.14	0.39 \pm 0.12	3.97 \pm 1.09	0.37 \pm 0.07
22:5 ω 6	0.12 \pm 0.06	0.09 \pm 0.13	0.23 \pm 0.18	0.17 \pm 0.16	nd	0.03 \pm 0.04
22:5 ω 3	0.14 \pm 0.18	0.01 \pm 0.02	0.19 \pm 0.03	0.07 \pm 0.04	0.09 \pm 0.05	0.10 \pm 0.09
22:6 ω 3	0.20 \pm 0.08	4.64 \pm 2.49	1.23 \pm 0.60	1.09 \pm 0.96	0.27 \pm 0.23	0.54 \pm 0.30
Σ saturated	8.61 \pm 4.67	9.47 \pm 8.27	5.93 \pm 1.70	16.72 \pm 2.47	8.43 \pm 0.15	15.90 \pm 2.68
Σ MUFA	40.99 \pm 15.44	15.96 \pm 8.77	30.37 \pm 4.3	42.46 \pm 8.97	48.34 \pm 4.51	47.86 \pm 4.93
Σ PUFA	13.9 \pm 8.5	59.29 \pm 17.39	29.81 \pm 3.97	27.49 \pm 9.08	14.27 \pm 2.15	27.68 \pm 4.00
Σ branched	6.15 \pm 8.72	0.98 \pm 0.89	0.97 \pm 0.34	2.01 \pm 0.45	1.81 \pm 2.56	0.48 \pm 0.10
Σ NMI	12.21 \pm 7.66	1.61 \pm 0.57	25.93 \pm 3.18	12.78 \pm 2.13	11.85 \pm 1.6	8.94 \pm 0.96
Not identified or <0.01	30.36 \pm 37.32	10.64 \pm 44.85	33.4 \pm 11.55	12.08 \pm 21.06	27.22 \pm 10.02	8.03 \pm 11.76
total lipids (mg.g ⁻¹ dwt)	46.3 \pm 22.1		14.0 \pm 1.6		189.7 \pm 53.8	

n.d.- not detected