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Ana Colaço, Catarina Prieto, Ana Martins, Miguel Figueiredo, Virginie Lafon, et al.. Seasonal variations in lipid composition of the hydrothermal vent mussel *Bathymodiolus azoricus* from the Menez Gwen vent field. *Marine Environmental Research*, 2009, 67 (3), pp.146. 10.1016/j.marenvres.2008.12.004 . hal-00501989

HAL Id: hal-00501989

<https://hal.science/hal-00501989>

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Accepted Manuscript

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PII: S0141-1136(08)00261-4
DOI: [10.1016/j.marenvres.2008.12.004](https://doi.org/10.1016/j.marenvres.2008.12.004)
Reference: MERE 3310

To appear in: *Marine Environmental Research*

Received Date: 16 April 2008
Revised Date: 11 December 2008
Accepted Date: 15 December 2008

Please cite this article as: Colaço, A., Prieto, C., Martins, A., Figueiredo, M., Lafon, V., Monteiro, M., Bandarra, N.M., Seasonal variations in lipid composition of the hydrothermal vent mussel *Bathymodiolus azoricus* from the Menez Gwen vent field, *Marine Environmental Research* (2008), doi: [10.1016/j.marenvres.2008.12.004](https://doi.org/10.1016/j.marenvres.2008.12.004)

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6 Running Head:

7 Seasonal variation lipids vent mussel

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ABSTRACT:

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

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

1. INTRODUCTION

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

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

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

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

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

2.3 Fatty acid analyses

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

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

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

2.4 FA structural verification

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

prepared by re-dissolving in 500 μ l of 2-amino-2-methylpropanol (FLUKA) heating overnight at 180°C. After cooling, the reaction mixture was dissolved in 5 ml of dichloromethane and washed twice with distilled water. The dichloromethane solution was dried with Na₂SO₄ and evaporated under a stream of nitrogen flow at room temperature. The residue was dissolved in n-hexane for analysis by gas chromatography–mass spectrometry (GC-MS).

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

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

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

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

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

2.6 Statistical analysis

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

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

3. RESULTS

3.1 Lipid classes

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

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

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

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

3.2 Fatty acids

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

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

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

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

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

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

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

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

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

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

3.4 Satellite Data

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

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

4. DISCUSSION

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

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

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

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

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

4.1 Fatty acid composition:

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

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

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

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

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

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

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

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

5. CONCLUSION

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

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

6. ACKNOWLEDGEMENTS:

The authors wish to acknowledge the crews of the RV 'L'Atalante and the RV 'Arquipélago', as well as the 'VICTOR 6000' teams for their field support. We would like to thank Prof. F. Barriga, who served as the chief scientist for the mission SEAHMA I. MODIS satellite data was obtained from NASA/GSFC and processed at the Department of Oceanography and Fisheries of the University of the Azores (DOP/UAç). IMARDOP/UAz is Research and Development Unit #531 and Associated Laboratory #9 funded by the Portuguese Foundation for Science and Technology (FCT) through pluri-annual and programmatic funding schemes (FEDER, POCI2001, FSE) and by the Azores Directorate for Science and Technology (DRCT). This research is a contribution of project PDCTM/P/MAR/15281/1999/ SEAHMA – Seafloor and sub-seafloor hydrothermal modeling in the Azores sea (POCI-POSI-FEDER). OPALINA (PDCTE/CTA/49965/2003) Projects.

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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.

Fig.2

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

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