Marine Environmental Research 67 (2009) 146–152

Contents lists available at ScienceDirect

Marine Environmental Research

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

Seasonal variations in lipid composition of the hydrothermal vent mussel *Bathymodiolus azoricus* from the Menez Gwen vent field

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ARTICLE INFO

Article history:
Received 16 April 2008
Received in revised form 11 December 2008
Accepted 15 December 2008

Keywords:
Menez Gwen
Bathymodiolus azoricus
Seasonality
Lipids
Fatty acids
Biomarkers
Hydrothermal activity

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 ω7 MUFA (monounsaturated fatty acids), whereas ω6 PUFA (polyunsaturated fatty acids) and NMI (non-methylene-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.

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, 840 m), since surface particles are estimated to sink at a rate of about 100 m per day (Gage and Tyler, 1991). The mussel *Bathymodiolus azoricus* dominates communities associated with deep-sea hydrothermal vents in the Azores Triple Junction (ATJ) region, at depths ranging from 840 m at Menez Gwen vent field to 2300 m 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-Médioni 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 (Dalsgaard 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

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doi:10.1016/j.marenvres.2008.12.004

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 18:4ω3; 20:3ω3; 22:5ω3; 22:6ω3 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.

2. Materials and methods

2.1. Sampling

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

2.2. Lipid extraction

Total lipids were extracted according to the Bligh and Dyer method (1959) as modified by White et al. (1979). The lipids were then separated into different fractions using solid phase extraction chromatography in silica gel columns (Isolute® SPE Columns). Non-polar lipid components were separated with 7.5 ml of diethyl ether–hexane (1:1). The medium polar lipid fraction (mainly glycolipids) was extracted with 7.5 ml of acetone and the high polar lipid fraction (mainly phospholipids) was recovered with 15 ml of methanol. The absolute value of each lipid fraction 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 min. Then, it was raised at a rate of 4 °C/min up to 220 °C, and maintained at 220 °C for 25 min 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 μl 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 Na_2SO_4 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 (Finningan Polaris Q mass spectrometer system - Thermo Electron Corporation, MA, USA). A splitless injection was performed with 1 μl of sample. The carrier gas was helium at 10 psi. A 25 m × 0.25-mm id x 0.25 μm HP-5® (Hewlett–Packard) column was used. The GC–MS conditions for DMOX derivatives elution were as follows: started with 2 min at 90 °C, followed by a 5 °C min^{-1} ramp up to 280 °C over 20 min.

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 OC3 M (O'Reilly et al., 2000) and long-wave SST (LW-SST) (Franz, 2006) algorithms, respectively. Regular daily MODIS images were obtained from the Ocean Color 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).