



$\delta^{13}\text{C}$ signature of hydrothermal mussels is related with the end-member fluid concentrations of H_2S and CH_4 at the Mid-Atlantic Ridge hydrothermal vent fields

Ana COLAÇO¹, Frank DEHAIRS², Daniel DESBRUYÈRES³,
Nadine LE BRIS³ and Pierre-Marie SARRADIN³

(1) IMAR- Department of Oceanography and Fisheries - University of Azores - 9900 Horta Portugal

Fax: (351) 292292659. E mail: acolaco@dop.horta.uac.pt

(2) Vrije Universiteit Brussel, Department of Analytical Chemistry (ANCH), Pleinlaan 2, B-1050 Brussels Belgium.

(3) 3 IFREMER Centre de Brest - DRO/EP - BP70, 29280 Plouzané, France

Introduction

Stable isotopic ratios for carbon are useful tools for revealing trophic interactions in a well-known and delimited ecosystem. The $\delta^{13}\text{C}$ isotopic signature is an indicator of the consumed food substrate(s), since $\delta^{13}\text{C}$ remains relatively unchanged between successive trophic levels ($\delta^{13}\text{C}$ becomes enriched by about 1 ‰ between each trophic level (Conway et al., 1994)). This paper tests a novel application of stable isotope ratios, a hypothesis first proposed by Trask & Van Dover (1999). These authors suggested that the nutritional response of mussels and their endosymbionts to environmental conditions (H_2S and CH_4 availability) can be discerned by comparing the concentrations of sulphide and methane in hydrothermal fluids and the stable isotopic composition of mussels at the ridge scale.

Trask & Van Dover (1999) reported differences in isotopic composition of mussels at the Lucky Strike vent field scale, and hypothesize a direct relation between the relative abundance of the endosymbionts in mussel gill tissues and the chemistry of the environment. These authors also found that the relative abundance of methanotroph endosymbionts ranged from 6 to 15% of in gill tissues of Lucky Strike mussels containing both endosymbionts. Distel et al. (1995), studying the coexistence of both methanotrophic and thiotrophic symbionts in mussels from Snake Pit, stated that thioautotrophic symbionts were considerably more abundant.

A study of nutritional relationships at several MAR vent communities (Colaço et al., 2002) using the stable carbon and nitrogen isotope approach revealed that the $\delta^{13}\text{C}$ signature of mussels was quite variable between and within hydrothermal vent fields, with values ranging from -24 to -36 ‰. This observation led us to consider a possible

relationship with hydrothermal fluid chemistry at the vent field scale. The largest $^{13}\text{C}/^{12}\text{C}$ discrimination originates during fixation of carbon by autotrophs and depends on the type of enzymes involved and on other factors such as diffusion distance, concentration gradients and temperature (Fisher, 1995). For heterotrophic organisms, $\delta^{13}\text{C}$ signature is mainly a function of the isotopic composition of the food source and, secondarily of a variety of processes which discriminate against one isotope over another during uptake and incorporation of the carbon (Fisher, 1995).

It has been well documented that MAR mussels host both thio- and methanotrophic symbionts (Robinson et al., 1999 for *Bathymodiolus puteoserpentis* Von Cosel et al., 1994; Fiala et al., 1996 for *B. azoricus* Von Cosel et al., 1999). Bacterial endosymbionts sustain chemolithoautotrophic primary production requiring H_2S and methanotrophic primary production requiring CH_4 provided by vent fluid and O_2 present in ambient seawater. The aerobic sulphur oxidizing bacteria (i.e. thioautotrophic bacteria) require both oxygen and sulphide sources (Jannasch, 1995), and use the Calvin Benson cycle to fix CO_2 . Robinson & Cavanaugh (1995) report that the carbon fixing enzyme for *B. thermophilus* Kenk & Wilson, 1985 endosymbiotic bacteria is Rubisco I. Assuming the same enzymatic pathway in MAR mussel symbionts, it is expected that mussels harbouring only sulphide-oxidizing symbionts will have a $\delta^{13}\text{C}$ value centred around -33 ‰ (Trask & Van Dover, 1999).

Methanotrophs, which belong to methylotrophic bacteria, subdivide into three types with their own metabolic pathway (types I, II and X). These bacteria require O_2 and CH_4 , the latter being energy and carbon sources (Fisher et al., 1987). According to Pond et al. (1998), mussels from Lucky Strike should have a $\delta^{13}\text{C}$ signature ranging from

-20 to -28‰ if deriving their carbon only from methanotrophic symbionts.

Mussels are present at all Mid Atlantic Ridge (MAR) hydrothermal vent fields (Menez Gwen, Lucky Strike, Rainbow, Broken Spur, Snake Pit, Logatchev) over a wide depth range (800 to 3400 m), with the exception of the TAG vent field (26°08'N; 3600 m). They belong to at least two species: (1) *Bathymodiolus azoricus* present at Menez Gwen (37°51'N; 800 m), Lucky Strike (37°18'N; 1700 m), Rainbow (36°13'N; 2500 m); (2) *Bathymodiolus puteoserpentis* present at Snake Pit (23°22'N; 3400 m) and Logatchev (14°45'N; 3000 m). The specific status of the mussels from Broken Spur (29°10'N; 3100 m) is still questioned (Von Cosel et al., 1999) despite the identification of individuals belonging to two species on the basis of molecular techniques (Jollivet et al., 1998; Maas et al., 1999).

At present, there are no available data on chemical conditions in the mussel habitat at the different MAR vent fields. For the purpose of this ridge-scale study, we used concentrations of sulphide and methane in end-member (high-temperature) hydrothermal fluid to characterize the mussel habitat in each vent field. The warm, diffusely-venting fluids found in the mussel habitat do not necessarily result from a straightforward dilution of end-member fluids. Their chemistry can also be altered by complex chemical and biological interactions. However, we sought only to develop a first-order approximation of the relative availability of sulphide and methane in the different vent fields, in order to relate habitat chemistry to average stable isotope signatures. At all known MAR (with the exception of Lost City) hydrothermal fields, variable but significant concentrations of methane (see Table 1) and hydrogen sulphide (reviewed in Desbruyères et al., 2000) have been observed.

Material and methods

Samples for this study were collected in June 1994, August 1997 and July 1998 by the submersible "Nautile" (samples taken by grab during the DIVA 1 & 2, MARVEL, FLORES and PICO cruises), and in July 1997 by the submersible "Alvin" during the MAR97 cruise. On board ship, ninety-nine mussels from the different samples were dissected for separation of muscle, which was deep-frozen (-20 or -80°C). Back in the laboratory, samples were then freeze-dried and reduced to powder for the $\delta^{13}\text{C}$ analyses. Statistical comparisons used the Spearman correlation (Siegel, 1988).

Results

There was a strong relationship (Table 1 and Fig. 1a and b) between the chemical composition of the end-member hydrothermal fluids (e.g. the concentration of methane and sulphide) and the mean $\delta^{13}\text{C}$ signals of the mussel tissues in a given population at the vent field scale. The $\delta^{13}\text{C}$ values

Table 1. Endmember concentrations of methane and sulphide in the MAR vent fields and corresponding $\delta^{13}\text{C}$ signature of hydrothermal mussels. Data used on figure 1. **a)** Colaço et al., 2002; **b)** Charlou et al., 2000 *Chemical geology*, 171: 49-75; **c)** Charlou et al., submitted *Geochimica et Cosmochimica Acta*; **d)** James et al., 1995 *Geochimica et Cosmochimica Acta*, **59**: 651-659; **e)** Campbell et al., 1988 *Journal of Geophysical Research* **93**: 4537-4549; Jean-Baptiste et al., 1991 *Earth and Planetary Science Letters*, 106: 17-28; Rudnicki & Elderfield, 1992 *Earth and Planetary Science Letters*, 113: 307-321.

	Hydrothermal field	H ₂ S(mM) (mean± SD) (n)	CH ₄ (mM) (mean± SD) (n)	$\delta^{13}\text{C}_a$ (‰). (mean± SD*) (n**)
MG	Menez Gwen ^b	1,6±0.2 2	1,7±0.27 2	-24,22±1.25 8
LS1	Lucky Strike ^b 1 ($\delta^{13}\text{C}$ -20)	2,45±0.64 5	0,645±0.29 5	-22,31±3.07 11
LS2	Lucky Strike ^b 2 ($\delta^{13}\text{C}$ -30)	2,84±0.31 11	0,49±0.17 11	-30,16±1.21 41
Rb	Rainbow ^c	1,75±0.75 3	2,35±0.15 3	-26,64±1.57 25
BS	Broken Spur ^d	9,3±1.7 2	0,06 1	-35,98±1 2
SP	Snake Pit ^e	4,4±1.7 4	0,078±0.032 2	-34,59±1.59 4
Lg	Logatchev ^d	1 1	2,1 1	-21,36±6.08 8

of the mussel tissues and CH₄ concentrations both decreased sharply in relation to increasing sulphide concentration at the sampled MAR vent fields (Fig. 1). There were significant correlations between the H₂S concentration values of the end-member fluid and $\delta^{13}\text{C}$ mussel isotopic signatures, and the H₂S and CH₄ end-member fluid concentrations ($\alpha < 0.05$), while the correlation between the CH₄ end-member fluids concentrations and the $\delta^{13}\text{C}$ mussel isotopic signatures was notable, but not significant at the 5% level ($0.06 > \alpha > 0.05$).

Discussion

These observations suggest that the availabilities of sulphide and methane have a direct influence on the relative contribution of the different symbionts and therefore the ratio of organic carbon derived by the host from the activity of the two types of symbionts. As a consequence, differences in the relative contribution of the two symbionts to host nutrition would be responsible for the field scale variation of mussel $\delta^{13}\text{C}$ signatures. No ontogenic variation was evident in the data because all analysed mussels were larger than 1cm total length.

These results are independent of the species considered: *Bathymodiolus azoricus* in Menez Gwen, Lucky Strike and Rainbow vent fields, *Bathymodiolus puteoserpentis* in Snake Pit and Logatchev vent fields, or *Bathymodiolus* spp. in Broken Spur vent field. Within the Lucky Strike vent field, the isotopic composition of the mussels is bimodal and reflects local heterogeneity of fluid composition (Trask & Van Dover, 1999; Colaço et al., 2002). For our Lucky Strike

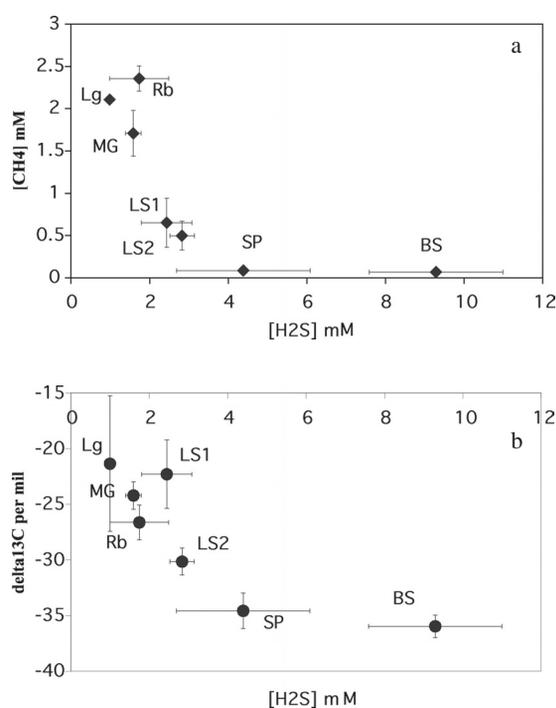


Figure 1. Relationship between end-member fluid composition (methane and sulphide) and mussel $\delta^{13}\text{C}$ for Mid-Atlantic Ridge Vent fields. **a** : end-member methane concentration vs. end-member hydrogen sulphide concentration. **b** : $\delta^{13}\text{C}$ signature of mussels' tissues vs. end-member sulphide concentration. Bars represent ± 1 standard deviation. Lg: Logatchev; Rb: Rainbow; MG: Menez Gwen; LS: Lucky Strike; SP: Snake Pit; BS: Broken Spur.

samples, we separated populations of mussels belonging to the “-20 $\delta^{13}\text{C}$ group” (LS1) from those belonging to the “-30 $\delta^{13}\text{C}$ group” (LS2), and separately considered the related end-member fluid compositions. Mussels collected at Snake Pit and Broken Spur exhibit the most negative $\delta^{13}\text{C}$ signatures (as low as -38‰) indicative of thiotrophic endosymbionts (Trask & Van Dover, 1999). At these vent fields the H_2S concentrations in the vent fluids are the highest and the methane concentrations the lowest (Fig. 1 a & b). Conversely, mussels coming from Logatchev, Menez Gwen, Rainbow and Lucky Strike1, sites with a high methane content, exhibit less negative $\delta^{13}\text{C}$ signatures (-25‰) indicative of endosymbionts more dependant on methanotrophy. Despite the paucity of information on the $\delta^{13}\text{C}$ ratios of the methane sources at MAR vent fields, the observed $\delta^{13}\text{C}$ values agree with expected values, when considering the published isotope fractionation values for methanotrophic bacteria (Jahnke et al., 1999), and taking the TAG (Charlou et al., 1996), Lucky Strike (-13‰), and Menez Gwen (-19‰) (Charlou et al., 2000) methane $\delta^{13}\text{C}$ values as representative for MAR sites.

The evidence for a close adaptation of Mytilid mussels to specific environments is strong. Clearly, these mussels are well adapted to thrive in environments where the ratio of sulphide over methane is highly variable regionally and even locally. The hosting of two physiologically distinct

bacteria within their tissues may provide the latter with a greater flexibility to exploit carbon and energy sources in a fluctuating environment while minimizing intersymbiont competition (Distel et al., 1995).

The amount of organic matter that each type of bacterial symbiont produces will depend on the relative availability of H_2S and CH_4 substrates. Symbiont activity will be regulated by the ratio of the energy source (H_2S or CH_4) and respective carbon source (CO_2 and CH_4).

Our results support the hypothesis that the relative availability of energy sources to the endosymbionts can regulate the carbon isotopic signature of the MAR mussels. As a consequence, $\delta^{13}\text{C}$ of mussel tissue should reflect the different proportions of the two symbionts' activity in the mussel gills not only at the vent field scale as stated by Trask & Van Dover (1999) but also at the ridge scale.

In the future we will be conducting laboratory experiments in which the relative concentration of the H_2S and CH_4 in the mussel environment is controlled. With this type of experiment we hope to better understand the response of mussel stable isotope signatures to H_2S and CH_4 availability.

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