

# Mixotrophy in the deep sea: a dual endosymbiotic hydrothermal mytilid assimilates dissolved and particulate organic matter

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**ABSTRACT:** *Bathymodiolus azoricus* mussels thrive 840 to 2300 m deep at hydrothermal vents of the Azores Triple Junction on the Mid-Atlantic Ridge. Although previous studies have suggested a mixotrophic regime for this species, no analysis has yet yielded direct evidence for the assimilation of particulate material. In the present study, tracer experiments in aquaria with <sup>13</sup>C- and <sup>15</sup>N-labelled amino acids and marine cyanobacteria demonstrate for the first time the incorporation of dissolved and particulate organic matter in soft tissues of vent mussel. The observation of phytoplanktonic tests in wild mussel stomachs highlights the occurrence of *in situ* ingestion of sea-surface-derived material. Particulate organic carbon fluxes in sediment traps moored away from direct vent influence are in agreement with carbon export estimates from the surface ocean above the vents attenuated by microbial degradation. Stable isotope composition of trapped organic matter is similar to values published in the literature, but is enriched by +7‰ in <sup>13</sup>C and +13‰ in <sup>15</sup>N, relative to mussel gill tissue from the Menez Gwen vent. Although this observation suggests a negligible contribution of photosynthetically produced organic matter to the diet of *B. azoricus*, the tracer experiments demonstrate that active suspension-feeding on particles and dissolved organic matter could contribute to the C and N budget of the mussel and should not be neglected.

**KEY WORDS:** *Bathymodiolus azoricus* · Particulate and dissolved material · Nitrogen and carbon assimilation · Deep sea · Hydrothermal vent · Mussel

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## INTRODUCTION

Menez Gwen (MG), Lucky Strike (LS) and Rainbow (RB), 3 hydrothermal vent sites identified at depths ranging from 800 to 2400 m on the Mid-Atlantic Ridge (MAR) Azores Triple Junction (ATJ), are colonised by large beds of the mussel *Bathymodiolus azoricus* (Desbruyères et al. 2001). Nutritional requirements and energy budgets of animals depending exclusively

on chemoautotrophy at the numerous sulphide-hydrocarbon cold seeps and hydrothermal vents worldwide have been well characterised (Childress & Fisher 1992). In contrast, the role of heterotrophy remains poorly understood in potential mixotrophic species, such as mytilids of the genus *Bathymodiolus*, inhabiting these environments.

At the 3 ATJ vent sites, mussel soft tissue carbon and nitrogen natural stable isotope (SI) signature variations

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occurring between vent fields and between sites within a single vent field range from  $-35.6$  to  $-14.7$ ‰ for  $\delta^{13}\text{C}$  and from  $-15.8$  to  $+0.2$ ‰ for  $\delta^{15}\text{N}$  (Trask & Van Dover 1999, Colaço et al. 2002a). SI signatures in *Bathymodiolus azoricus* thus indicate large nutritional differences between adult mussels. *B. azoricus* hosts a dual symbiosis with both methane- and sulphide-oxidising bacteria (Duperron et al. 2006) supporting part of the C requirements of the mussel (Riou et al. 2008). Differences in C isotopic signatures can thus be partly explained by varying proportions of symbiont populations in the gills due to differing  $\text{H}_2\text{S}$  and  $\text{CH}_4$  concentrations at vent sites (Colaço et al. 2002b, Duperron et al. 2006). However, we are currently unable to explain how the dense mussel beds of *B. azoricus* are sustained at MG, given the high respiration rates measured in aquaria (Martins et al. 2008) and the low  $\text{H}_2\text{S}$  and  $\text{CH}_4$  concentrations around the mussel clumps reported to date.

*In situ* analyses below mussel beds might reveal higher  $\text{H}_2\text{S}$  and  $\text{CH}_4$  availability, but these mussels probably also rely on the uptake of particulate and dissolved organic matter (POM and DOM, respectively; Martins et al. 2008). Particle retention and clearance rate mechanisms are common to bivalve families with differing morphological properties (oyster, clam and mussel; Ward et al. 1993) and should also operate in *Bathymodiolus azoricus*. Other members of the Bathymodiolinae suspension-feed on both hydrothermal-derived and phytoplankton-derived organic matter (Le Pennec & Hily 1984, Le Pennec & Priour 1984, Fiala-Médioni et al. 1986, Le Pennec 1988, Page et al. 1990, 1991), and the palps of the seep mussel '*B.* *childressi*' are capable of selective sorting (Pile & Young 1999). However, particle selection is species-dependent (Ward et al. 2003).

*Bathymodiolus azoricus* has a functional feeding groove in the gill and well developed labial palps, and its intestine is coiled (Von Cosel et al. 1999), contrasting with the straight digestive tract observed in *B. thermophilus*. These physiological properties of the northern MAR species could be an indication for greater dependency on ingested particles. It is hazardous to extrapolate observations made on 1 species to another, in particular when habitat properties differ, and direct evidence that *B. azoricus* assimilates POM is still missing. Mytilidae can take up free amino acids from surrounding seawater and can use them for osmoregulation or as a nutritional supplement (Ferguson 1982, Bishop et al. 1983). Bathymodiolinae are no different, taking up and assimilating amino acids from ambient seawater (Fiala-Médioni et al. 1986, Lee et al. 1992, Lee & Childress 1995), although no observations have previously been made on *B. azoricus*.

The phospholipid fatty acid (PLFA) composition of *Bathymodiolus azoricus* tissues indicates assimilation

of sulphate-reducing bacteria and phytoplankton, which appears variable in time (Colaço et al. 2007, 2009). In aquaria, this mussel is also capable of increasing mucus production in the gill tissue in the presence of POM and the absence of methane and sulphide (Bettencourt et al. 2008), which would aid particulate uptake. This adaptability to particulate feeding probably also occurs in the wild. Hence, a dynamic energy budget model suggests greater reliance on suspension-feeding in small-sized animals (Martins et al. 2008). A photosynthetically-derived component in the diet of *B. azoricus* larvae and post-larvae is suggested by the enriched  $\delta^{15}\text{N}$  signature, up to  $+4.2$ ‰ (Trask & Van Dover 1999). Natural SI signatures, as well as PLFA compositions of adult mussel tissues, suggested that sea-surface-derived particles made little contribution to the overall nutrition of *B. azoricus* (Colaço et al. 2002b, 2009). However, according to the low turnovers measured in '*B.* *childressi*' (Dattagupta et al. 2004), bulk tissue SI analysis may not be suited for the detection of short-term variations in the nutrition of an animal. Moreover, the lipid composition of bivalves depends on the physiological state of the animal (reproduction, stress; e.g. Nalepa et al. 1993) and can vary widely over the year (Colaço et al. 2009). A seasonal input of sea-surface-derived organic matter might well not be detected in the PLFA, depending on the time of mussel collection and on the extent of the phytoplankton bloom.

Hydrothermal plumes contain up to a few thousand times more bacteria than the deep sea (Karl 1995). In the RB vent field, neutrally buoyant plumes carry mineral particles to which numerous bacteria are attached (German & Von Damm 2003). Compared to nearby deep seawater, hydrothermal fluids collected at MAR ultramafic-hosted vents are clearly enriched in organic compounds that originate from a mixture of biogenic and abiogenic processes (Konn et al. 2009). In addition, there is increasing evidence that diffuse effluents from sub-seafloor regions adjacent to high-temperature vents harbour significant bacterial communities involved in  $\text{CH}_4$  production,  $\text{NO}_3^-$  reduction and  $\text{H}_2\text{S}$  oxidation (Santelli et al. 2008). Diffuse fluids perfusing through mussel beds may thus provide them with suspended bacteria that might also be a major source of DOM (Lang et al. 2006). Sarradin et al. (1999) reported dissolved organic carbon (DOC) concentrations in MG and LS off-vent bottom water to range between  $143$  and  $169 \mu\text{mol l}^{-1}$ . Such values are up to 3 times more concentrated than those reported for North Atlantic Deep Water below  $1000$  m ( $48^\circ\text{N}$ ,  $13^\circ$ ,  $41^\circ$  and  $48^\circ\text{W}$ ; and  $32^\circ\text{N}$ ,  $64^\circ\text{W}$ , Hansell & Carlson 1998). DOC concentrations in diffuse fluids at MAR mussel beds were even higher ( $247 \mu\text{M}$ ,  $n = 17$ , Sarradin et al. 1999) than in local off-vent bottom seawater. Unfortunately, to our

knowledge, no data on dissolved organic nitrogen (DON) concentrations in diffuse fluids below the mussel beds have been published yet. Although few measurements of DON levels have been made at vents (Karl 1995), amino acid concentrations (which would be the primary form of DON available) were between 0.5 and 4.2  $\mu\text{M}$  above seep mussel beds (Lee et al. 1992).

Even though they thrive close to hydrothermal chimneys, mussel beds are not permanently under chimney influence: this depends on current direction and speed. Sediment traps located close to RB and LS vents were thus observed to be under vent influence only 10% of the time of particle collection (Khripunoff et al. 2000, 2001), leaving 90% of the time for sinking pelagic material to reach the mussel beds. C export from the sea surface (photosynthetic primary production) is enhanced, even in summer (low production), at MG, LS and RB vents, due to the enhanced productivity at the northern side of the Azores Front (Macedo et al. 2000, Huskin et al. 2004). Some observations on the composition of exported matter at 200 m (Huskin et al. 2004) match the sediment trap observations at LS and RB (Khripunoff et al. 2000, 2008). Also, the phytoplankton blooms (mainly in spring and autumn) could be traced down to the vent sites (Khripunoff et al. 2008). Sea-surface primary production might thus represent a seasonal nutritional supplement to *Bathymodiolus azoricus*.

In the present study we performed  $^{13}\text{C}$  and  $^{15}\text{N}$  tracer experiments to test the potential of *Bathymodiolus azoricus* for N and C assimilation from POM and DOM and to calculate incorporation rates in aposymbiotic muscle tissues. Suspension-feeding activity *in situ* was investigated by stomach content analysis and by comparing the natural abundance of  $^{13}\text{C}$  and  $^{15}\text{N}$  in tissues of wild specimens to the  $^{13}\text{C}$  and  $^{15}\text{N}$  content of particles sedimenting near mussel beds.

## MATERIALS AND METHODS

**Enriched nutrient preparations and analytical methods.** Lyophilised *Agmenellum quadruplicatum* cyanobacteria, labelled with 98%  $^{13}\text{C}$  and 96–99%  $^{15}\text{N}$  (CNLM-455, Cambridge Isotope Laboratories), were used as the particulate food source. After rehydration, the particle size spectrum, as measured with a Coulter LS230 analyser (Beckman Coulter) ranged from 0.8 to 6.5  $\mu\text{m}$  diameter, with 2 maxima at 1.7  $\mu\text{m}$  (cyanobacteria) and 4.7  $\mu\text{m}$  (probably aggregates). The amounts of labelled organic C and N released as dissolved material during rehydration were estimated from the differences in C and N contents (analysed with a Thermo-Flash 1112 Elemental Analyzer [EA]) between dry cyanobacteria and rehydrated, filtered (Whatman

GF/F) cells (unlabelled ULM-2177, Cambridge Isotope Laboratories). Thus, 15% of the N (98%  $^{15}\text{N}$ ) and 18% of the organic C (98%  $^{13}\text{C}$ ) were released as DOM. A free amino acid (FAA) mixture (Gly 20–25%, Ala 15–20%, Tyr 10–15%, Leu 5–10%, Lys 5–10%, Ser 5–10%, Thr 2–5%, Phe 1–5%, Pro 1–5%, Val 1–5%, Met <3%, Try <1%, Ile <1%, His <1%) labelled with 98%  $^{13}\text{C}$  and  $^{15}\text{N}$  (Campro Scientific) was used for the DOM uptake experiments. C and N molar ratios of the mixture were measured on an NA1500 (Carlo Erba) EA using acetanilide (Merck, 71.09% C and 10.36% N) as a standard.

DOC concentration in the 0.2  $\mu\text{m}$  filtered seawater used for tracer experiments was measured on a Shimadzu TOC 5000A. DON concentration was obtained by subtracting the amount of dissolved inorganic N ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ) from the total dissolved N quantified by digestion using a microwave oven (CEM Mars 5) equipped with Teflon PFA<sup>®</sup> sample vessels (Dafner et al. 1999).  $\text{NH}_4^+$  was quantified by the indophenol blue complex method,  $\text{NO}_3^-$  was determined using a Technicon<sup>™</sup> A-II model auto-analyser based on the sulphanilamide colourimetric method, and  $\text{NO}_2^-$  was considered negligible.

For natural and enriched SI analyses, lyophilised mussel tissues were ground to a fine powder using a mortar and pestle. Aliquots of tissue powder, or lyophilised particles, were weighed into silver cups (previously heated at 450°C for 4 h), acidified with a few drops of 5% HCl to remove any possible trace of carbonates, and re-dried overnight at 60°C. The analyses were performed on a Flash1112 EA coupled to a Delta V Isotope Ratio Mass Spectrometer via a ConFlo III interface (Thermo Finnigan). Tissue and particle C and N contents were assessed from the thermal conductivity detector (TCD) signal of the EA, using acetanilide as a standard. Enriched tissue SI ratios are expressed as atom% values, defined as:

$$A = [{}^{\text{H}}X / ({}^{\text{H}}X + {}^{\text{L}}X)] \times 100 [\%] \quad (1)$$

where X is the abundance of the element (C or N), and H and L the heavy and light isotopes, respectively. Control and wild mussel natural SI ratios are expressed relative to C and N conventional references (Vienna PeeDee Belemnite [VPDB] limestone and atmospheric  $\text{N}_2$ , respectively) as  $\delta$  values:

$$\delta X = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 10^3 [\text{‰}] \quad (2)$$

where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . Maximum SDs between aliquots of the same tissue sample were 0.50‰ for  $\delta^{15}\text{N}$  and 0.25‰ for  $\delta^{13}\text{C}$ . The International Atomic Energy Agency (IAEA) standards ( $^{13}\text{C}$ : IAEA-CH-6 or IAEA-309B;  $^{15}\text{N}$ : IAEA-N1 or IAEA-N2, of natural abundance and enriched, respectively) followed the same analytical procedure as the unknown samples.

**Aquarium tracer experiments.** Mussel cages were deployed and loaded with approximately 400 *Bathymodiolus azoricus* next to the PP30 marker at the MG vent field in August 2006 (37° 51' N, 32° 31' W, 817 m), during the MOMARETO cruise on RV 'Pourquoi Pas?' (see Riou et al. 2008). In May 2007, 1 cage was released and retrieved by the Portuguese RV 'Arquipélago' 20 min later. Ten 'wild' mussels (62.9 to 69.2 mm shell length, SL) were dissected on board; tissues were frozen for later lyophilisation on land. The remaining cage specimens were transferred to seawater at 9°C for a 14 h transit to the LabHorta land-based laboratory. The outsides of the mussels were scrubbed clean of visible material and rinsed with chilled seawater. Mussels were then placed in refrigerated aquaria with aerated seawater to which CH<sub>4</sub> and H<sub>2</sub>S were added (Riou et al. 2008). They were maintained in these conditions for 38 d before tracer experiments started.

The tracer experiments were carried out over a period of 20 d on a total of 42 adult mussels (59.7 to 72.4 mm SL). Three different 4 l tanks, containing 14 mussels each, were filled with aerated, 0.2 µm filtered seawater. The water was changed every 2 d and monitored daily for temperature (7.8–9.6°C), pH (7.1–8.6) and O<sub>2</sub> saturation (median: 45%). One tank was used as a control (no additions) while another tank was supplied with 13 mg l<sup>-1</sup> rehydrated <sup>13</sup>C- and <sup>15</sup>N-labelled *Agmenellum quadruplicatum* every other day after water replacement. Particles were maintained in suspension with an air-lift pump. The third tank was supplied with 9 mg l<sup>-1</sup> of the <sup>13</sup>C- and <sup>15</sup>N-labelled FAA mixture at each water change. At the end of the incubations, mussels were placed into filtered seawater for 24 h to allow gut clearance. They were then dissected, and the gill, mantle, total muscle and remaining tissues were rinsed in distilled water (to remove non-incorporated label), frozen and lyophilised.

Dry tissues were weighed to the nearest mg before homogenisation for SI analyses. A gill index (GI) was calculated from each specimen's gill tissue and rest of body (total soft tissue – gill) dry weights according to the following formula:

$$GI = [\text{gill tissue dry weight (g)} / \text{rest of body dry weight (g)}] \times 100 \quad (3)$$

**Wild mussels and sediment trap studies.** During the 'Marvel' cruise (early September 1997) stomachs of 2 mussel specimens from Menez Gwen PP30 (91 mm SL) and Lucky Strike 'Bairro Alto' (129 mm SL) were fixed in 5% buffered formalin. The stomach contents were examined using a Philips XL30 scanning electron microscope (Philips Analytical). As we chose to focus on the nutrition of MG mussels, we only show the material observed in the stomach of the MG specimen.

Sedimenting particles were collected at MG for a period of 10 d during the August 1997 'Marvel' cruise by sediment traps moored just above the MG site (next to the PP30 marker) and a site 2 km away from the vents (Desbruyères et al. 2001). Pelagic particles were also collected during sampling periods of 14 d by long-term moorings 2 km off the RB site in 1997 to 1998 (Khripounoff et al. 2001) and 2001 to 2002 (Khripounoff et al. 2008).

**Data treatment and statistics.** Muscle tissue C and N incorporation (C<sub>inc</sub> and N<sub>inc</sub> in µmol g<sup>-1</sup> dry tissue) were calculated as:

$$X_{inc} = [(A_{exp} - A_{control}) \times X_{tissue}] / (A_{substrate} - A_{control}) \quad (4)$$

where  $A_{exp}$  is the <sup>13</sup>C or <sup>15</sup>N atom% measured in the mussel after the tracer experiment,  $A_{control}$  is the median <sup>13</sup>C or <sup>15</sup>N atom% measured in the mussels kept for 20 d in the control tank (N = 10),  $X_{tissue}$  is the C or N content of the tissue analysed (µmol g<sup>-1</sup> tissue) and  $A_{substrate}$  is the <sup>13</sup>C or <sup>15</sup>N atom% of the substrate used for the experiment. Incorporation rates were obtained by dividing net incorporations by the duration of the tracer experiment.

Differences in condition index between fresh and enriched mussels were analysed by non-parametric Kruskal-Wallis (ANOVA) on raw data, due to the low number of replicates.

## RESULTS

### Aquarium filter-feeding experiments

No mussels were lost during the tracer experiments, although control and DOM-experiment mussels had a significantly lower GI than wild individuals (Kruskal-Wallis  $p < 0.01$ , N = 20, Fig. 1). The GIs of specimens from the 3 experiments were not significantly different from each other (Kruskal-Wallis  $p = 0.67$ , N = 30), nor was the difference between the POM-experiment GI (although highly variable) and wild mussel GI significant (Kruskal-Wallis  $p = 0.11$ , N = 20; Fig. 1).

After 4 d of exposure, labelled N and C from FAA were mainly found in the gill tissue, the other tissues being considerably less enriched (data not shown). In contrast, feeding with cyanobacteria resulted in C and N incorporation first in tissues including the digestive system and pseudo-faeces, then in gill, but less in muscle and mantle tissues (data not shown).

The muscle tissue isotopic signatures of control mussels (mean ± SD:  $\delta^{15}\text{N} = -8.6 \pm 0.2\%$ ,  $\delta^{13}\text{C} = -29.3 \pm 0.5\%$ , N = 10,) were similar to wild specimens ( $\delta^{15}\text{N} = -8.4 \pm 1.1\%$ ,  $\delta^{13}\text{C} = -29.6 \pm 0.5\%$ , N = 10). Values displayed in parentheses in Table 1 account for the concurrent incorporation of seawater unlabelled DOM; fil-

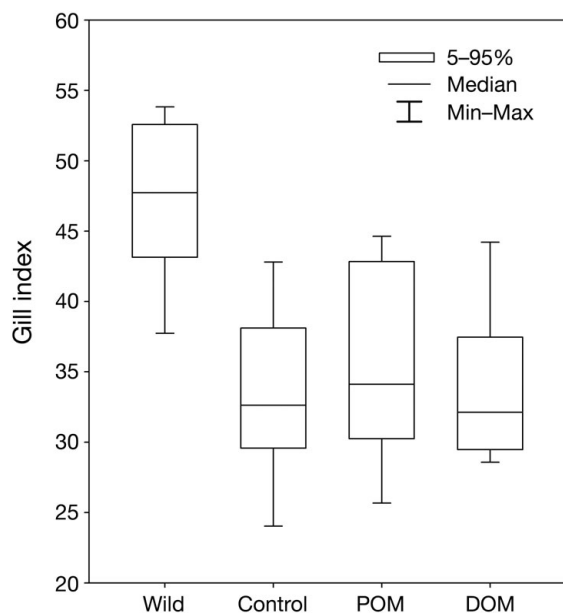


Fig. 1. *Bathymodiolus azoricus*. Gill index of adult mussels from the wild and from different aquarium experiments. POM: mussels fed with particulate food (rehydrated lyophilised cyanobacteria); DOM: mussels fed with a mix of dissolved amino acids; Control: mussels kept in filtered seawater (20 d, N = 10 for each condition, 59.7 to 72.4 mm shell lengths)

tered seawater contained  $4.6 \mu\text{mol l}^{-1}$  of DON and approximately  $250 \mu\text{mol l}^{-1}$  of DOC, but we were unable to determine the fraction of FAA. If we consider that this DON consists exclusively of FAA with natural isotopic composition ( $0.364\%$   $^{15}\text{N}$ ,  $1.112\%$   $^{13}\text{C}$ ), with the same C:N molar ratio as for the FAA mix (3.9), filtered seawater itself could supply a maximum of  $4.6 \mu\text{mol l}^{-1}$  DON and  $17.9 \mu\text{mol l}^{-1}$  DOC in the form of FAA. This would result in the following  $A_{\text{substrate}}$  (Eq. 4):  $A_{\text{FAAmix}} = 92.5$  and  $92.6\%$  and  $A_{\text{cyanobacteria}} = 87.4$  and  $91.8\%$  for N and C, respectively, instead of 98% when only the added tracer is taken into account.

The FAA tracer experiment thus resulted in median muscle incorporation rates of  $2.5 \pm 0.9 \mu\text{mol N g}^{-1}$  dry muscle  $\text{d}^{-1}$  and  $7.0 \pm 2.8 \mu\text{mol C g}^{-1}$  dry muscle  $\text{d}^{-1}$  (neglecting seawater DOM assimilation, N = 13,  $\pm$  interquartile range), while median muscle rates of  $1.0 \pm 0.6 \mu\text{mol N g}^{-1}$  dry muscle  $\text{d}^{-1}$  and  $3.1 \pm 2.2 \mu\text{mol C g}^{-1}$  dry muscle  $\text{d}^{-1}$  (N = 13,  $\pm$  interquartile range) were measured when feeding the mussels with cyanobacteria.

Assuming that DON and DOC incorporations relate linearly with concentrations, regardless of the nature of the DOM, we estimated the fraction of new muscle N and C originating from the 15% DON and 18% DOC released during cyanobacteria rehydration. A maxi-

mum of 22% N ( $0.04 \text{ mmol}$ ) and 42% C ( $0.23$  to  $0.24 \text{ mmol}$ ) could have been incorporated from this DOM. This means that at least 78% of the N and 58% of the C would have been incorporated from particulate matter.

Muscle tissue total molar C:N ratio was  $3.9 \pm 0.1$  (N = 28, median  $\pm$  interquartile range). The C:N incorporation ratio was  $3.3 \pm 0.2$  (N = 14, median  $\pm$  interquartile range) for the experiment with cyanobacteria (with a C:N = 6.1), while it was significantly lower ( $2.8 \pm 0.1$ , N = 14, median  $\pm$  interquartile range) in mussels incubated with the FAA mix (with a C:N = 3.9).

### Observations at vent fields

Tissue isotopic signatures of cage mussels collected in May 2007 (Fig. 2) were very close to tissues  $\delta^{15}\text{N}$  (mean  $\pm$  SD:  $-11.82 \pm 1.49$ , N = 35,) and  $\delta^{13}\text{C}$  ( $-31.09 \pm 3.05$ , N = 38) reported for mussels collected at the same location more than 10 yr earlier (MG PP30, Colaço et al. 2002a). No bacteria were found among the material inside the stomachs. Instead, mineral skeletons with diameters reaching  $40 \mu\text{m}$  were observed: a planktic foraminifera and a centric diatom of the suborder Coscinodiscineae, possibly a species of *Thalassiosira*, were present in the stomach of the MG specimen (Fig. 3). A silicoflagellate (probably of the genus *Dichtyochoa*) and remains of centric diatoms were found in the stomach of the LS specimen (data not shown).

Table 2 displays the fluxes and isotopic signatures of particulate N and organic C in trapped material collected 2 km away from MG or RB vents at the time mussels were collected for stomach content analysis. The table also shows the results for a 1 yr pelagic particle collection between 2001 and 2002 (Khripounoff et al. 2008). Most of the samples were preserved in formalin (values italicised in Table 2), which alters C isotope ratios depending on the nature of the preserved material (e.g. Edwards et al. 2002). Interpretations based on particle  $\delta^{13}\text{C}$  signatures should therefore be evaluated critically. However, a subtraction of  $0.5\%$  should accurately correct the  $\delta^{15}\text{N}$  values for formalin alteration (Arrington & Winemiller 2002; bold, Table 2). The trap material was low in N as revealed by C:N ratios ranging from 7.2 to 9.6. Roughly 1% of the pelagic flux at 1500 m off RB was composed of N, with an isotope signature that varied little on an annual scale ( $+2.2\%$  in 2001 to 2002, Table 2) but considerably between years ( $+4.3\%$  in 1997). Although particulate organic C (POC) content varied from 4 to 11% with a maximum in late spring coinciding with a maximum in total mass flux (Khripounoff et al. 2008), its isotopic signature varied little intra- and inter-annually ( $-23.0\%$ ).



Table 1. *Bathymodiolus azoricus*. Characteristics and muscle C and N net incorporation from enriched re-hydrated lyophilised cyanobacteria or free amino acids in each specimen sampled after 4 and 20 d experiments in aquaria. Values in parentheses account for the concurrent incorporation of seawater unlabelled dissolved organic matter (DOM); values in **bold** were used to calculate the incorporation rates. GI: gill index,  $X_{\text{muscle}}$ : measured C or N content of the tissue analysed ( $\mu\text{mol g tissue}^{-1}$ ),  $A_{\text{exp}}$ :  $^{13}\text{C}$  or  $^{15}\text{N}$  atom% measured in the mussel after the tracer experiment,  $A_{\text{control}}$ :  $^{13}\text{C}$  or  $^{15}\text{N}$  atom% measured in the control mussel,  $N_{\text{inc}}$  and  $C_{\text{inc}}$ : net C and N tissue incorporation

Days	Shell length (mm)	Dry muscle weight (g)	Total dry body weight (g)	GI	Nitrogen			Carbon				
					$X_{\text{muscle}}$	$A_{\text{exp}}$	$(A_{\text{exp}} - A_{\text{control}})$	$N_{\text{inc}}$	$X_{\text{muscle}}$	$A_{\text{exp}}$	$(A_{\text{exp}} - A_{\text{control}})$	$C_{\text{inc}}$
							( $\mu\text{mol g}^{-1}$ dry tissue)					( $\mu\text{mol g}^{-1}$ dry tissue)
Cyano-bacteria	62.9	0.192	0.904	36.7	8817	0.39558	0.0318	<b>2.9 (3.2)</b>	32639	1.1063	0.0329	<b>10.9 (11.7)</b>
(Total input in 20 d:	61.1	0.169	1.079	32.9	7856	0.47013	0.1063	<b>8.5 (9.6)</b>	31715	1.1644	0.0909	<b>29.4 (31.5)</b>
7.9 mmol C	63.9	0.164	0.982	43.3	8516	0.42683	0.0630	<b>5.5 (6.2)</b>	31876	1.1353	0.0618	<b>20.1 (21.6)</b>
1.1 mmol N)	70.0	0.187	0.999	40.5	7009	0.48087	0.1171	<b>8.4 (9.4)</b>	27126	1.1644	0.0910	<b>25.5 (27.0)</b>
	68.0	0.206	1.231	29.8	8251	0.53642	0.1726	<b>14.6 (16.4)</b>	32304	1.2189	0.1454	<b>48.5 (51.4)</b>
	67.2	0.218	1.327	30.4	8642	0.59146	0.2277	<b>20.2 (22.6)</b>	33587	1.2532	0.1797	<b>62.3 (66.0)</b>
	67.7	0.150	1.425	35.1	8532	0.59584	0.2320	<b>20.3 (22.7)</b>	33758	1.2535	0.1800	<b>62.7 (66.5)</b>
	63.2	0.198	1.171	25.2	8374	0.65022	0.2864	<b>24.6 (27.6)</b>	34017	1.3121	0.2386	<b>83.7 (88.8)</b>
	65.6	0.144	0.650	44.7	8504	0.69308	0.3293	<b>28.7 (32.2)</b>	32434	1.3553	0.2819	<b>94.3 (100.0)</b>
	63.5	0.115	0.688	32.3	8141	0.51538	0.1516	<b>12.6 (14.2)</b>	31263	1.1989	0.1255	<b>40.5 (42.9)</b>
	62.0	0.173	0.625	42.4	8419	0.49215	0.1284	<b>11.1 (12.4)</b>	31907	1.1880	0.1145	<b>37.7 (40.0)</b>
	63.5	0.144	0.750	44.2	8418	0.52410	0.1603	<b>13.8 (15.5)</b>	32188	1.2165	0.1430	<b>47.5 (50.3)</b>
	59.8	0.178	0.801	33.1	8642	0.59475	0.2310	<b>20.4 (22.9)</b>	32455	1.2686	0.1952	<b>65.3 (69.3)</b>
FAA mix	68.7	0.145	1.542	31.7	9139	0.53114	0.1673	<b>15.7 (16.6)</b>	35656	1.2101	0.1367	<b>49.7 (52.8)</b>
(Total input in 20 d:	62.7	0.187	0.852	49.1	7948	0.44877	0.0850	<b>6.9 (7.3)</b>	30323	1.1368	0.0634	<b>19.6 (20.8)</b>
9.7 mmol C	61.4	0.273	1.306	32.2	8445	0.41601	0.0522	<b>4.5 (4.8)</b>	32816	1.1115	0.0381	<b>12.7 (13.5)</b>
2.5 mmol N)	70.2	0.275	1.726	29.4	8537	0.87287	0.5091	<b>44.5 (47.2)</b>	33588	1.4217	0.3482	<b>120.7 (126.8)</b>
	68.0	0.232	1.174	33.5	8487	0.96967	0.6059	<b>52.7 (55.8)</b>	32528	1.5234	0.4499	<b>151.0 (156.7)</b>
	69.4	0.220	0.921	44.9	8558	0.64688	0.2831	<b>24.8 (26.3)</b>	32479	1.2888	0.2153	<b>72.2 (75.8)</b>
	69.4	0.206	1.165	38.2	8522	0.99878	0.6350	<b>55.4 (58.7)</b>	33011	1.5034	0.4299	<b>146.4 (153.9)</b>
	63.3	0.220	1.021	29.5	8684	0.96546	0.6017	<b>53.5 (56.7)</b>	33431	1.5007	0.4273	<b>147.4 (154.9)</b>
	64.2	0.176	0.993	37.2	8478	1.06865	0.7049	<b>61.2 (64.9)</b>	32249	1.5818	0.5083	<b>169.1 (177.7)</b>
	64.3	0.220	1.380	28.5	8636	1.09258	0.7288	<b>64.5 (68.3)</b>	33826	1.5945	0.5211	<b>181.8 (191.1)</b>
	63.8	0.186	1.271	29.7	8531	0.84901	0.4852	<b>42.4 (44.9)</b>	33784	1.4020	0.3285	<b>114.5 (120.3)</b>
	62.1	0.211	1.007	33.6	8524	0.93166	0.5679	<b>49.6 (52.5)</b>	33039	1.4855	0.4120	<b>140.4 (147.6)</b>
	59.7	0.202	0.762	30.7	8499	0.83222	0.4684	<b>40.8 (43.2)</b>	32343	1.3923	0.3189	<b>106.4 (111.8)</b>

Table 2. C:N ratio, organic carbon and nitrogen fluxes (particulate organic carbon: POC; particulate nitrogen: PN; in  $\text{mg m}^{-2} \text{d}^{-1}$ ) and isotopic composition from sediment trap particles collected at various distances from active vents, distances (m) above bottom (a.b.), depth and dates (Desbruyères et al. 2001, Khrpounoff et al. 2008). Samples preserved in formalin are in *italics*, and corrected values are in **bold**. MG: Menez Gwen, RB: Rainbow, nm: no more material available for analysis

Location	Distance a.b. (depth) (m)	Date	POC	$\delta^{13}\text{C}$ (‰)	PN	$\delta^{15}\text{N}$ (‰)	C:N
2 km off MG	2 (840)	Late Aug 1997	3.5	nm	0.5	nm	9.6
1 m from MG	2 (840)	Late Aug 1997	10.4	-27.8	0.8	-2.1	15.6
2 km off RB	200 (1950)	Late Aug 1997	1.2	<i>-23.0 ± 1.0</i>	0.2	<i>4.8 ± 0.3 / <b>4.3</b></i>	8.5
2 km off RB	400 (1550)	Jul 2001 (min.)	2.6	-23.1	0.4	<b>2.7 / <b>2.2</b></b>	7.9
2 km off RB	400 (1550)	May 2002 (max.)	7.5	-23.1	1.2	<b>2.7 / <b>2.2</b></b>	7.2

## DISCUSSION

*Bathymodiolus azoricus* mussels thriving at around 800 m depth at the MG hydrothermal vent site have the capacity to recover from the decompression stress experienced during cage recovery and to survive maintenance at atmospheric pressure, providing an invaluable advantage for a study of their nutrition under controlled conditions. The combination of *in vitro* SI tracer experiments with *in situ* analyses yields evidence that *B. azoricus* has the potential to incorporate vent- and sea-surface-derived dissolved and particulate organic C and N. Natural SI composition has often been used to establish relative trophic positions within a food web, considering that  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of consumers are systematically heavier than those

of their diet (e.g. Colaço et al. 2002b). However, applying a mixing model using natural SI signatures to infer the contribution of the 4 different food sources available to *B. azoricus* (2 symbionts, POM and DOM) requires knowing their SI signature. This is complex to obtain, as the symbionts are intra-cellular, and POM and DOM result from a mix of multiple processes in an unstable environment submitted to seasonal deposition of particles derived from sea-surface primary production (SSPP). Tracer experiments with stable or radio-isotopes are thus needed to assess the role of suspension-feeding in the nutrition of deep-sea hydrothermal mytilids. In our tracer experiments, the muscle tissue was chosen for the low probability of contamination with non-assimilated FAA or cyanobacteria (as opposed to the mantle, which stores pseudofaeces, the gill, which traps particles in mucus, or the digestive tract), or with cyanobacteria ingested by haemocytes (Bettencourt et al. 2008), which could lead to over-estimations in the net incorporation calculations.

The highest C assimilation rate measured in the muscle tissue in our experiments reached  $0.50 \mu\text{mol C g}^{-1} \text{dry tissue h}^{-1}$  with  $302 \mu\text{M}$  DOC from dissolved free amino acids (DFAA), and  $0.29 \mu\text{mol C g}^{-1} \text{dry tissue h}^{-1}$  with  $263 \mu\text{M}$  POC cyanobacteria. These values were considerably higher than the incorporation rates measured in the muscle tissue of *Bathymodiolus azoricus* maintained in the same experimental conditions with  $\text{CH}_4$  or  $\text{H}_2\text{S}$ . Maximal C incorporation from  $\text{CH}_4$  in *B. azoricus* muscle tissues after the 38 d maintenance period was  $0.022 \mu\text{mol C g}^{-1} \text{dry wt h}^{-1}$  with  $30 \mu\text{M}$  methane ( $0.23 \mu\text{mol C g}^{-1} \text{dry wt h}^{-1}$  in gill tissue, Riou et al. 2008). The methane uptake rate is linear up to  $200 \mu\text{M}$  (P. Dando pers. obs.), and high methane concentrations above  $300 \mu\text{M}$  are inhibitory for symbionts of '*B. childressi*' exhibiting high methane consumption rates (Kochevar et al. 1992). Thus, with 7 times more methane (i.e. a methane concentration closer to the DOC and POC used in the present experiments) a rate of  $0.15 \mu\text{mol C g}^{-1} \text{dry wt h}^{-1}$  could have been reached, which is on the same order

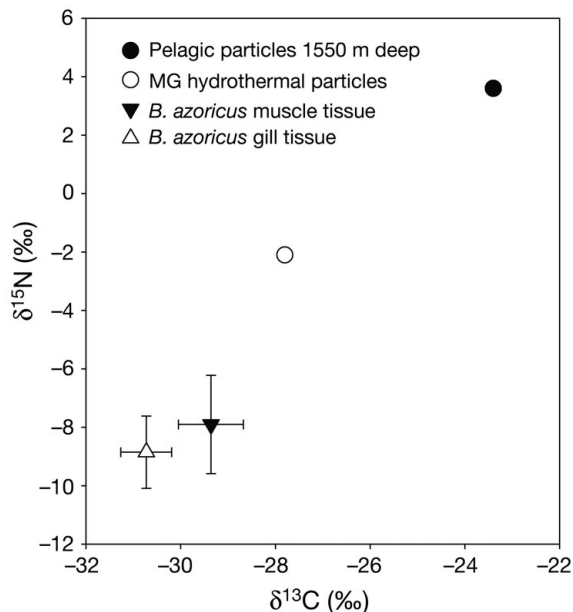


Fig. 2. C and N isotopic composition of sedimenting sea-surface material (●), Menez Gwen (MG) vent-derived particles (collected in 1997, ○) and *Bathymodiolus azoricus* gill (Δ) and mantle (▼) tissues (N = 10) from the MG (PP30) May 2007 cage

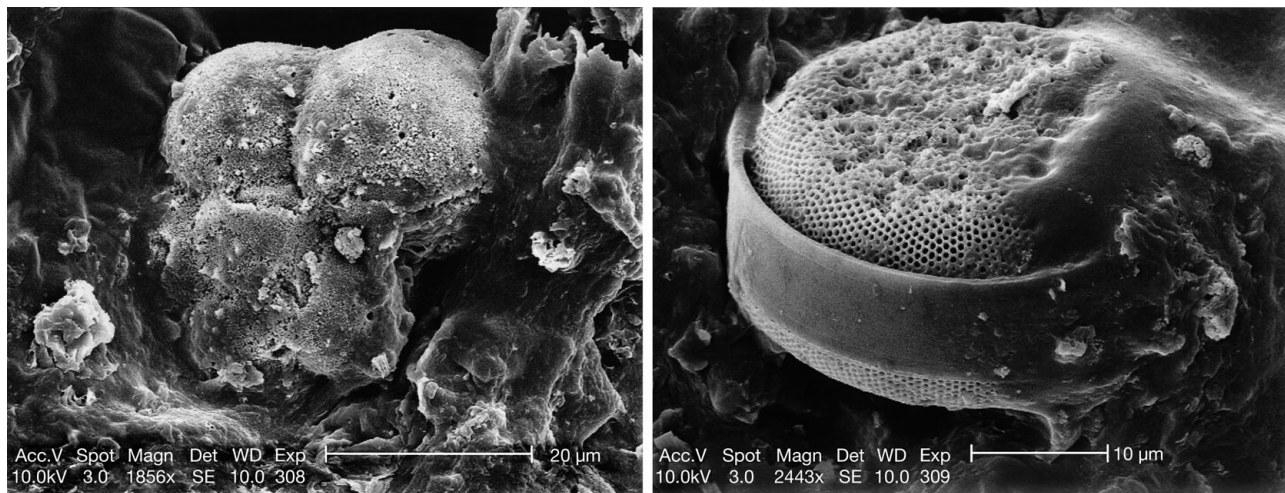


Fig. 3. *Bathymodiolus azoricus*. Images of the structures observed by scanning electron microscopy in preserved stomachs of a specimen collected at Menez Gwen (left: undetermined foraminifera test; right: *Thalassiosira* diatom frustule)

as the rates obtained with POC and DOC. Maximal C incorporation from  $\text{CO}_2$  and sulphide was  $0.15 \mu\text{mol C g}^{-1}$  dry wt  $\text{h}^{-1}$  in *B. azoricus* muscle tissue with  $6 \mu\text{M}$  sulphide ( $0.37 \mu\text{mol C g}^{-1}$  dry wt  $\text{h}^{-1}$  in the gill, Riou et al. 2008). Assuming that 5 moles of S are needed to reduce 1 mole of  $\text{CO}_2$  in SOX bacteria (Kelly & Kuenen 1984), a sulphide concentration 250 times higher than  $6 \mu\text{M}$  would have to be used to compare the SOX assimilation rates to the rates obtained with the current DOC and POC experiments. A linear uptake rate would theoretically have resulted in the incorporation of  $37.5 \mu\text{mol C g}^{-1}$  dry wt  $\text{h}^{-1}$ , without taking into account the high toxicity of  $\text{H}_2\text{S}$  used at  $1.5 \text{ mM}$ .

The  $\text{CH}_4$  and  $\text{CO}_2$  incorporation rates in *Bathymodiolus azoricus* gill tissue seem particularly low, compared to other incorporation rates measured on the MOX symbiotic '*B. childressi*' and the SOX symbiotic *Solemya reidi* (which were converted to  $\mu\text{mol g}^{-1}$  dry wt  $\text{h}^{-1}$  using a dry:wet tissue weight ratio of 0.162 for the sake of comparison, Martins et al. 2008). Indeed, maximal C incorporation from  $\text{CH}_4$  in '*B. childressi*' gill tissue was  $37 \mu\text{mol g}^{-1}$  dry wt  $\text{h}^{-1}$  with  $200 \mu\text{M}$   $\text{CH}_4$  (Lee & Childress 1995), and in *S. reidi* gill,  $\text{CO}_2$  was incorporated at a rate of  $28 \mu\text{mol C g}^{-1}$  dry wt  $\text{h}^{-1}$  with  $100 \mu\text{M}$   $\text{H}_2\text{S}$  (Lee & Childress 1994). In addition to the fact that the substrate ( $\text{CH}_4$  and  $\text{H}_2\text{S}$ ) concentrations used in the experiments with *B. azoricus* were considerably lower than those used with '*B. childressi*' and *S. reidi*, some *B. azoricus* individuals lose symbionts following the physiological stress of recovery to the surface, although reacquisition occurs (Kádár et al. 2005). This was observed by rough FISH bacterial area estimates (Riou et al. 2008). Nevertheless, as there is also evidence of DNA damage after mussel collection (Pruski & Dixon 2003), a recovery period is required

before the mussels can be expected to behave in a physiologically normal manner; hence the need for a maintenance period.

Maximal N incorporation rates in '*Bathymodiolus childressi*' and *S. reidi* gill tissues from inorganic N were  $3.4$  and  $3.1 \mu\text{mol g}^{-1}$  dry wt  $\text{h}^{-1}$ , respectively, with  $50 \mu\text{M}$   $\text{NH}_4^+$  (Lee & Childress 1994, 1995). In mantle tissue of '*B. childressi*', a rate of  $0.49 \mu\text{mol N g}^{-1}$  dry wt  $\text{h}^{-1}$  was measured (Lee & Childress 1995). The highest N assimilation rates measured in our experiments with DFAA and cyanobacteria were comparable to this last value, reaching  $0.16$  and  $0.09 \mu\text{mol N g}^{-1}$  dry tissue  $\text{h}^{-1}$ , respectively. This observation indicates that DON and particulate organic N (PON) can be an important supplement to the nutrition of *B. azoricus*. This has already been suggested for '*B. childressi*', for which Pile & Young (1999) mentioned that a rate of  $0.74 \mu\text{mol N g}^{-1}$  dry tissue  $\text{h}^{-1}$  could be attained by filter feeding.

### Assimilation of DOM

FAAs, simple sugars, proteins and polysaccharides are among the most common organic compounds found as exudates (Hellebust 1974). The organic C pool in marine waters is thus mainly composed of DFAAs and carbohydrates (Münster 1993). A study of the water overlying '*Bathymodiolus childressi*' mussel beds found total DFAA concentrations between  $0.5$  and  $4.2 \mu\text{M}$  (Lee et al. 1992, our Table 3). The DFAA mix used in the present tracer experiment included 11 out of the 14 amino acids detected over '*B. childressi*' beds (and an additional 3 amino acids). Table 3 displays the individual concentrations found over mussel beds and those used in the tracer experiment.



Table 3. Free amino acids used during the dissolved organic matter (DOM) tracer experiment, compared to the average amino acid concentration detected over a '*Bathymodiolus childressi*' bed (Lee et al. 1992). The molecular weights (MW) of the 98%  $^{13}\text{C}$ - and  $^{15}\text{N}$ -enriched amino acids were re-calculated, together with their minimal and maximal concentrations in the seawater (according to their proportion in the mixture given by the supplier in %). The most abundant amino acids found near mussel beds are in **bold**; (-) absent from the mix

Amino acid	MW (g mol <sup>-1</sup> )	Min. ( $\mu\text{M}$ )	Max. ( $\mu\text{M}$ )	'B.' <i>childressi</i> beds ( $\mu\text{M}$ )
Aspartate	-	-	-	0.04
<b>Glutamate</b>	-	-	-	<b>0.16</b>
Arginine	-	-	-	0.01
Histidine	163.8	-	0.55	0.03
Glycine	77.9	23.09	28.87	0.02
Alanine	92.9	14.53	19.37	0.08
Tyrosine	190.8	4.72	7.08	0.01
<b>Serine</b>	<b>108.9</b>	<b>4.13</b>	<b>8.26</b>	<b>0.11</b>
Threonine	123.9	1.45	3.63	0.07
Methionine	154.9	-	1.74	0.02
<b>Valine</b>	<b>122.9</b>	<b>0.73</b>	<b>3.66</b>	<b>0.10</b>
<b>Phenylalanine</b>	<b>174.8</b>	<b>0.51</b>	<b>2.57</b>	<b>0.48</b>
<b>Isoleucine</b>	<b>137.9</b>	-	<b>0.65</b>	<b>0.14</b>
<b>Leucine</b>	<b>137.9</b>	<b>3.26</b>	<b>6.53</b>	<b>0.11</b>
Lysine	153.8	2.93	5.85	-
Proline	120.9	0.74	3.72	-
Tryptophan	216.7	-	0.42	-

A high total DFAA concentration (9 mg l<sup>-1</sup>) was used to label the mussel tissues significantly within 20 d tracer experiments, and to simulate a DOC concentration (302  $\mu\text{M}$ ) close to those measured above *Bathymodiolus azoricus* beds (216 to 647  $\mu\text{M}$  at MG, Sarradin et al. 1999) under the assumption that all DOC available at mussel beds is in the form of DFAA (which is obviously not the case, but gives a basis to estimate the maximal C incorporation rate by the mussels from the DOC present *in situ*). The DOC concentrations measured by Sarradin et al. (1999) might have been over-estimated, since the DOC concentration in control off-vent bottom seawater (143  $\mu\text{M}$ ) was 3 times higher than the 44 to 45  $\mu\text{M}$  reported by Hansell & Carlson (1998) for deep Atlantic water. However, they are the only data available to date for MAR diffuse vents.

Lee et al. (1992) reported amino acid uptake rates for '*Bathymodiolus childressi*' of 1.2 to 2.4  $\mu\text{mol C g}^{-1}$  wet tissue h<sup>-1</sup> and 0.6 to 0.8  $\mu\text{mol N g}^{-1}$  wet tissue h<sup>-1</sup> when provided with 10  $\mu\text{M}$  glycine and alanine, respectively. In our labelling experiments, *B. azoricus* was provided with ~26  $\mu\text{M}$  glycine and ~17  $\mu\text{M}$  alanine (representing 40% of the total dissolved amino acids added to the seawater, and around 100  $\mu\text{M}$  DOC). If the rates of incorporation were linear and similar in both mussel species, we would expect an uptake rate of at least 7.2

and 2.9  $\mu\text{mol g}^{-1}$  wet tissue h<sup>-1</sup> for C and N, respectively (not taking into account the other amino acids in solution). In muscle tissue, we found maximal incorporation rates of 0.52 and 0.16  $\mu\text{mol g}^{-1}$  dry tissue h<sup>-1</sup> for C and N, respectively (0.08 and 0.03  $\mu\text{mol C and N g}^{-1}$  wet tissue h<sup>-1</sup>, assuming a dry to wet weight conversion factor of 0.162; Martins et al. 2008). These incorporation rates are 2 orders of magnitude lower than the uptake rates expected from the study by Lee et al. (1992).

However, our rates are incorporation rates, not uptake rates. Amino acids can be catabolised to produce energy (releasing CO<sub>2</sub> and NH<sub>3</sub>). Incorporation rates will thus be lower than uptake rates due to catabolism of amino acids taken up, and also to the loss of amino acids across external membranes (excretion). Following Martins et al. (2008), we express the energetic loss by *Bathymodiolus azoricus* due to respiration as:

$$R = e^{2.69} W^{0.76} \text{ (in } \mu\text{mol C g}^{-1} \text{ dry weight h}^{-1}\text{)} \quad (5)$$

where  $W$  is the mussel dry body weight. According to this relationship, the individual in which we measured the highest C incorporation rate, having a body dry weight of 1.54 g (68.7 mm SL), would need to respire 3.6  $\mu\text{mol C g}^{-1}$  wet weight h<sup>-1</sup>, representing half of the uptake rate as estimated above (7.2  $\mu\text{mol C g}^{-1}$  wet tissue h<sup>-1</sup>) and leaving 3.6  $\mu\text{mol C g}^{-1}$  wet tissue h<sup>-1</sup> to be incorporated in the amino acid pools and organic matter of the whole mussel soft body.

Compared to gill, mantle and the rest of the tissues, muscle tissue displays the lowest rates of C and N incorporation into metabolite pools and tissue components that can be 10 times lower than the gill tissue incorporation rates (data not shown). This can explain part of the difference observed between the whole mussel soft body C incorporation rate of 3.6  $\mu\text{mol C g}^{-1}$  wet tissue h<sup>-1</sup> estimated from the uptake rates measured by Lee et al. (1992) and the maximal muscle incorporation rate of 0.08  $\mu\text{mol C g}^{-1}$  wet tissue h<sup>-1</sup> that we measured.

Furthermore, Lee & Childress (1995) reported whole '*Bathymodiolus childressi*' soft tissue assimilation rates of 0.62 and 0.48  $\mu\text{mol g}^{-1}$  wet tissue h<sup>-1</sup> for C and N, respectively, as obtained from incubation experiments amended with 50  $\mu\text{M}$   $^{13}\text{C}^{15}\text{N}$ -glycine (corresponding to 100  $\mu\text{M}$  DOC). Assuming that the mussels used by Lee & Childress (1995) in their study also had a 1.5 g body dry weight, their assimilation rates  $\text{g}^{-1}$  wet tissue would be about 6 times lower than the 3.6  $\mu\text{mol C g}^{-1}$  wet tissue h<sup>-1</sup> whole mussel soft body assimilation rate that we estimated using the uptake rates of Lee et al. (1992). C assimilation rates from glycine are low compared to the rates observed for  $^{13}\text{CH}_4$  assimilation (i.e. 1.79  $\mu\text{mol C g}^{-1}$  wet tissue h<sup>-1</sup>), but N assimilation rates are high relative to rates of  $^{15}\text{NH}_3$  assimilation of

0.18  $\mu\text{mol N g}^{-1}$  wet tissue  $\text{h}^{-1}$  (Lee & Childress 1995). However, decoupling of C and N assimilations from  $^{13}\text{C}^{15}\text{N}$ -glycine was reported, indicating that sources of C other than from glycine are used to assimilate amino N (Lee & Childress 1995). The ability to use stored C and the decoupling of C and N assimilation (as well as the preferential use of particular amino acids for osmoregulation) can result in a C:N ratio of assimilated organic matter well below the value of 4.0 in *B. azoricus* muscle tissue and of the DFAA mixture (3.3). Indeed, in the present tracer experiment, a particularly low C:N ratio (2.5) was measured in the organic matter formed from the assimilation of the FAA.

FAA uptake by epidermal transport can serve as an important nutritional supplement to *Mytilus* and *Modiolus* mussels, with Michaelis constants for uptake in the micromolar range (Wright 1982). Nevertheless, uptake rates measured under laboratory conditions may not reflect the situation in the wild, as it is likely that competition between marine metazoans and (heterotrophic) bacteria occurs for FAA uptake (Siebers 1982). Although we cannot exclude the presence of some heterotrophic bacteria in the seawater, the filtration of the water through 0.2  $\mu\text{m}$  porosity filters should have limited their presence. This raises the question of possible FAA incorporation by bacterial fauna attached to the mussel shells, or by endosymbionts of *Bathymodiolus azoricus*. Indeed, although the symbionts were reported to assimilate  $\text{CH}_4$  or  $\text{CO}_2$  in the presence of  $\text{H}_2\text{S}$  (Riou et al. 2008), their potential for mixotrophy was never tested. Gill tissues being the first to reveal FAA label incorporation agrees with the observations of Wright (1982) but could also indicate assimilation by the high amounts of symbionts in these tissues. SI tracer incorporation into bacterial PLFA biomarkers should help validate this hypothesis.

### Assimilation of POM

During the maintenance period, which precedes feeding experiments, alteration of gill epithelium and loss of endosymbionts is known to occur (Kádár et al. 2005, Riou et al. 2008). Nevertheless, Riou et al. (2008) observed active (although reduced) endosymbiotic populations in mussel specimens after the maintenance period, as well as a stabilisation of the GI already after 30 d. The fact that mussels fed with cyanobacteria had GI values not significantly different from wild mussels, as opposed to mussels from the control tank and the DOM experiment (Fig. 1), even suggests that gills had further adapted to suspension-feeding and recovered from the maintenance period.

The supply of cyanobacteria was equivalent to 12 and 75  $\text{mg m}^{-2} \text{d}^{-1}$  of N and C, respectively. Such a C

supply exceeds the annual maximal sinking flux measured at RB between 2001 and 2002 (Table 2) by 1 order of magnitude. Despite the large cyanobacterial C theoretically available in our experiment, only 1.4% of the input appeared to be incorporated by the muscle tissue. Maximal muscle tissue turnover, if assumed to be linear over time, would only reach 3.8 and 3.2% for N and C, respectively after 1 yr. This is around 10 times less than whole soft body SI turnovers calculated for '*Bathymodiolus childressi*' with no shell growth (Dattagupta et al. 2004). However, these SI turnovers were estimated for whole mussels, including the gill tissue that contained methane-oxidising symbionts. The values thus do not represent the mussel tissue C and N turnover, and comparison of our values to those of Dattagupta et al. (2004) is not straightforward.

Maximum incorporation rates of 0.089 and 0.306  $\mu\text{mol g}^{-1}$  dry muscle  $\text{h}^{-1}$  N and C, respectively, were measured in a *Bathymodiolus azoricus* individual with 61.1 mm SL and a whole body dry weight of 1.07 g (with energetic needs due to respiration of 2.7  $\mu\text{mol C g}^{-1}$  wet weight  $\text{h}^{-1}$ , according to Eq. 5; Martins et al. 2008). No rates of C incorporation are available for comparison from studies with bathymodiolids, since none were calculated during the  $^{14}\text{C}$ -tracer filter-feeding experiments with '*B.*' *childressi* and *B. thermophilus* (Page et al. 1990, 1991). Martins et al. (2008) estimated the POC requirements of *B. azoricus* of 10, 50 and 110 mm SL, assuming that filter-feeding was the only C source, as 0.05, 0.52 and 9.1  $\text{mg POC l}^{-1} \text{d}^{-1}$ , respectively. Applying an exponential fitting curve to this data, we calculate that our mussel, of 61.1 mm SL, would require 0.78  $\text{mg POC l}^{-1} \text{d}^{-1}$ . We added 1.6  $\text{mg l}^{-1} \text{d}^{-1}$  in the cyanobacteria experiment, which could have sustained growth of only 2 mussels of similar size.

Our POM tracer experiment tank contained 10 to 14 specimens, meaning that, although using POC concentrations 10 times higher than those measured *in situ*, our conditions were limiting for growth. Only 1.4% of the total POC input appears to have been incorporated into muscle tissues organic matter, but much more might have been used for energetic purposes, implying that the mussels must have been considerably depleting the POM inputs. These limiting conditions can also explain the low C and N incorporation rates observed.

According to the rates of DOM incorporation calculated from the DFAA mixture tracer experiment, the 6 and 47  $\mu\text{M}$  labelled DON and DOC, respectively, released by rehydration of the lyophilised cyanobacteria, would have led to maximum incorporation rates of 0.012  $\mu\text{mol N g}^{-1}$  dry muscle  $\text{h}^{-1}$  and 0.078  $\mu\text{mol C g}^{-1}$  dry muscle  $\text{h}^{-1}$ . These rates represent 13 and 27%, respectively, of the maximal incorporation rates measured in the POM tracer experiment. Thus, at least 87 and 73% of the N and C incorporated during the POM

tracer experiment were incorporated from particulate matter. These results suggest that most of the N and C were incorporated in the form of individual cyanobacterial cells or agglomerates (0.8 to 7.0  $\mu\text{m}$ ).

*Mytilus edulis* can retain particles with a minimal diameter size of 1  $\mu\text{m}$  (Jørgensen 1949), which corresponds to the minimal particle size in our tracer experiment. An apparent shift towards larger plankton with increasing mussel SL was observed in '*Bathymodiolus childressi*', with only the smaller specimens showing an ability to retain cyanobacteria (Pile & Young 1999). The results of our experiments, where the supplied food covered a wide range of particle size, demonstrate the capacity of *B. azoricus* for POM assimilation. Future work should focus on the relationship between particle size retention and mussel SL.

### In situ observations

The sediment trap POC flux at 840 m measured off MG in August 1997, when the Azores Front (AF) was located slightly south of the RB, LS and MG vents (between 33° 59' N, 32° 04' W and 34° 39' N, 31° 29' W; Schiebel et al. 2002) was 3.5 mg C m<sup>-2</sup> d<sup>-1</sup> (or 292  $\mu\text{mol C m}^{-2} \text{d}^{-1}$ , Table 2). SSPP measurements 1 mo earlier at the AF (located farther north at that time, 37° N, 32° W to 32° N, 29° W, Macedo et al. 2000) indicated that the water mass covering the vent area at the northern side of the AF was more productive than the southern water mass. Applying a Martin et al. (1987) relationship parametrising the attenuation of POC flux with depth, i.e.  $\Phi_{\text{POC}}(z) = \Phi_{\text{POC}}(z_0) \cdot [z/z_0]^{-b}$  (where  $\Phi$  is the flux,  $z$  the depth and  $b$  the attenuation on coefficient, we calculate a POC flux at 840 m ranging between 0.75 and 2.70 mg C m<sup>-2</sup> d<sup>-1</sup> (with  $z_0 = 100$  m and  $b$  varying between 0.7 and 1.3; Berelson 2001) from the new production estimated for the most productive water mass at the sea surface (12 mg C m<sup>-2</sup> d<sup>-1</sup>, Macedo et al. 2000). These estimates are slightly lower than the flux collected by the MG pelagic sediment trap. The latter was positioned 1.5 m above the bottom, and it is most likely that a large fraction of the collected particles came from sediment resuspended by bottom currents, leading to overestimation of the pelagic POC flux. In contrast, a trap covering the same period as the MG trap deployment, deployed 2 km away from RB at 1950 m (200 m above the seafloor, Khripounoff et al. 2001) recorded a POC flux of 1.2 mg C m<sup>-2</sup> d<sup>-1</sup> (Table 2), well within the range of the calculated POC flux using the Martin et al. (1987) relationship ( $0.27 < \Phi_{\text{POC}}(1950 \text{ m}) < 1.5$  mg C m<sup>-2</sup> d<sup>-1</sup>). These results confirm that the 3 vents benefit from the enhanced productivity of the water mass north of the AF.

C:N ratios of settling particles trapped outside MG and RB were relatively high, which is consistent with

the finding that the C:N ratio increases with depth (Huskin et al. 2004). The lowest C:N ratio (7.2, Table 2) of trapped pelagic particles was recorded in May 2002 during a peak of SSPP (Colaço et al. 2009). This low C:N ratio would point to an increased contribution of zooplankton and/or (cyano)bacteria relative to phytoplankton. Deposition of cyanobacteria probably originating from sinking faecal pellets produced by the overlying pelagic zooplankton community was already observed 650 m deep on the Louisiana slope (Pile & Young 1999). POC and particulate N (PN) isotopic signatures of SSPP material sinking in the RB area and collected in traps 1550 and 1950 m deep ( $\delta^{13}\text{C} = -23\%$  and  $2.2 < \delta^{15}\text{N} < 4.3\%$ , Table 2) are in the lower range of the isotopic signatures reported for POM sinking at 150 m in the North Atlantic Eastern Subtropical gyre (Rau et al. 1992). However, we cannot infer a trophic relationship between sinking particles and mussel tissues based on isotopic data alone, since MG PP30 mussel tissues are depleted by 6 to 7‰ and 2 to 3‰ in <sup>15</sup>N and <sup>13</sup>C, respectively, compared to particles sedimenting at the same location (Fig. 2).

The mussels analysed for stomach contents were collected in August 1997, when the AF was located slightly south of RB, LS and MG vents (between 33° 59' N, 32° 04' W and 34° 39' N, 31° 29' W; Schiebel et al. 2002). The presence of diatom and foraminifera tests in the stomach of the wild MG mussel investigated, as well as of silicoflagellates and diatoms in the specimen from the deeper LS vent site (data not shown), constitutes circumstantial evidence that *Bathymodiolus azoricus* living 840 to 1690 m deep ingest material of pelagic origin. North of the AF, diatoms and dinoflagellates dominate the sinking phytoplankton biomass at 200 m (86% of phytoplankton flux; Huskin et al. 2004), even though most of the phytoplanktonic C biomass in the photic zone is accounted for by picoplankton and flagellates. Live foraminifera were found at depths of at least 700 m on the northern side of the front (Schiebel et al. 2002). This is consistent with the observation that pelagic POM found in sediment traps close to RB can be rich in foraminifera and diatoms (Khripounoff et al. 2000, 2008).

The average SL of the wild mussels collected in May was 65.5 mm, and the average whole soft body dry weight and muscle and gill dry weights were 1.56, 0.25 and 0.50 g, respectively. An average mussel would thus use 32.2  $\mu\text{mol C h}^{-1}$  for respiration, according to Eq. (5) (Martins et al. 2008). At MG, the fluid diffusing through mussel beds had maximal sulphide concentrations of 62  $\mu\text{M}$ . In these conditions, the uptake rate was 13.6  $\mu\text{mol S g}^{-1} \text{dry gill h}^{-1}$  (P. Dando unpubl. data), and assuming that 5 mol of S are required to reduce 1 mol of CO<sub>2</sub> (Kelly & Kuenen 1984), this would allow a maximum fixation of 2.72  $\mu\text{mol C g}^{-1} \text{dry gill h}^{-1}$ , ex-

cluding any chemical oxidation of sulphide before it reaches the bacteria. An average 65.5 mm SL, wild *Bathymodiolus azoricus* could have obtained 1.36  $\mu\text{mol C h}^{-1}$  *in situ* through the utilisation of sulphide.

Since at the MG vent the  $\text{CH}_4\text{:H}_2\text{S}$  concentration ratio in end member fluids is around 1 (Desbruyères et al. 2000), a maximal  $\text{CH}_4$  concentration of 62  $\mu\text{M}$  could potentially have been diffusing through the mussel beds. Uptake of  $\text{CH}_4$  by isolated gills of freshly collected *Bathymodiolus azoricus* is linear with concentration, over the 14 to 200  $\mu\text{M}$  concentration range, and is in the region of 15  $\mu\text{mol g}^{-1}$  dry gill  $\text{h}^{-1}$  at 62  $\mu\text{M}$   $\text{CH}_4$  (P. Dando unpubl. data). Kochevar et al. (1992) measured that 70% of this uptake rate can be fixed by '*B. childressi*'s MOX symbionts, which would give a fixation rate of 10.5  $\mu\text{moles C g}^{-1}$  dry gill  $\text{h}^{-1}$ . A mussel with the average gill tissue dry weight used in our experiments could have obtained *in situ* 5.2  $\mu\text{mol C h}^{-1}$  from  $\text{CH}_4$  oxidation. Altogether, MOX and SOX symbiotic activities would cover 20% of the energetic needs of the mussel *in situ*.

A maximal DOC concentration of 647  $\mu\text{M}$  was measured above the mussel beds (Sarradin et al. 1999). According to Lee et al. (1992), a 10  $\mu\text{M}$  glycine concentration (corresponding to 20  $\mu\text{M}$  DOC) would lead to an uptake rate for '*Bathymodiolus childressi*' of 1.2  $\mu\text{mol C g}^{-1}$  wet tissue  $\text{h}^{-1}$  (or 7.4  $\mu\text{mol C g}^{-1}$  dry tissue  $\text{h}^{-1}$ ). If all of the DOC concentration measured by Sarradin et al. (1999) were also representative for our study, and if it is assimilated to the same extent as glycine, an uptake rate of 240  $\mu\text{mol C g}^{-1}$  dry tissue  $\text{h}^{-1}$  would be possible. A wild mussel, with 65.5 mm SL, a total soft body dry weight of 1.56 g, and a respiration rate of 32.2  $\mu\text{mol C h}^{-1}$  (Eq. 5; Martins et al. 2008) may thus assimilate approximately 374  $\mu\text{mol C h}^{-1}$ . We note that even if most of the DOC measured over mussel beds was refractory, resulting for instance in assimilable DOC concentrations 10-fold lower than the concentrations reported by Sarradin et al. (1999), it would still cover the energetic needs of a 1.56 g soft tissue wild mussel.

The organic particles associated with hydrothermal activity are also a potential organic C food source for *Bathymodiolus azoricus*: the concentrations are up to 938  $\mu\text{mol C m}^{-2} \text{d}^{-1}$  close to the MG vent; they can be approximated to 392  $\mu\text{mol C m}^{-2} \text{d}^{-1}$  at LS if a yearly median mass flux of 131  $\text{mg m}^{-2} \text{d}^{-1}$  is considered (with around 3% organic C), and 625  $\mu\text{mol C m}^{-2} \text{d}^{-1}$  at RB, at a yearly median mass flux of 500  $\text{mg m}^{-2} \text{d}^{-1}$  with a median of 1.25% organic C (Desbruyères et al. 2000, Khripounoff et al. 2008). If a density of 550  $\text{ind. m}^{-2}$  is considered at MG (Martins et al. 2008), a POC flux of 938  $\mu\text{mol C m}^{-2} \text{d}^{-1}$  would provide each mussel with 0.07  $\mu\text{mol C h}^{-1}$ , which appears negligible in comparison to the calculations from endosymbiosis and DOC

assimilation described above. However, tidal resuspension of sedimented organic matter could increase the availability of POM to the mussel beds.

While the POC would have a negligible impact on *Bathymodiolus azoricus*, Pile & Young (1999) mentioned a possible rate of N assimilation for '*B. childressi*' of 0.12  $\mu\text{mol N g}^{-1}$  wet tissue  $\text{h}^{-1}$  (= 0.99  $\mu\text{mol N g}^{-1}$  dry tissue  $\text{h}^{-1}$  using the 0.162 conversion factor used by Martins et al. 2008) by filter feeding. A 1.56 g wild mussel (10  $\text{mmol N}$  assuming 9% N tissue content) could thus obtain 1.54  $\mu\text{mol N h}^{-1}$  by feeding on particles, representing a daily N turnover of 0.37% (135% of the total N  $\text{yr}^{-1}$ ). Thus, POM could be an important N source for *B. azoricus*. However, very low PN fluxes were measured around MG and RB (0.2 to 1.2  $\text{mg N m}^{-2} \text{d}^{-1}$ , Table 2). At a mussel density of 550  $\text{ind. m}^{-2}$  (Martins et al. 2008), a PN flux of 86  $\mu\text{mol N m}^{-2} \text{d}^{-1}$  would provide each mussel with 0.006  $\mu\text{mol N h}^{-1}$ , which is 257 times less than the maximal assimilation rate in a 1.56 g wild mussel.

The trophic structure of hydrothermal vent communities depicted by Gebruk et al. (1997) pointed towards the possibly important but unknown contribution of photosynthetic organic C and heterotrophic microbial production. Particularly for bathymodiolid mussels, reliance on symbiosis can be combined with suspension feeding and DOM uptake. The relative availability and composition of the different nutritional sources is largely unknown and is likely to vary in time. Although it is difficult to assess their relative contribution to mussel biomass, this study demonstrates that bathymodiolids from northern MAR vents are potentially under the influence of seasonally pulsed SSPP, and are able to ingest and assimilate filtered material as well as DOM from the surrounding water. Only limited information has been published on the DOM available to *Bathymodiolus azoricus* mussel beds. Future studies should focus on allochthonous DOM composition, C and N contents and SI signatures in diffuse venting areas below mussel beds.

With respect to particulate-feeding, the vent mussels resemble most deep-sea bivalves living away from the vents in the Atlantic. Sokolova (2000) stated that 'suspension feeding bivalves are even more typical of the Atlantic macrobenthos than deposit feeding molluscs', although the majority of these (80%) had total weights of <0.5 g. The rain of small particles derived from surface production is of great significance in the flux of organic matter into the deep ocean. The sinking organic matter is thus thought to form a labile food source used for reproductive growth (Tyler 1988). Ripe gonads were identified in *Bathymodiolus azoricus* collected at MG in late January 2003, followed by the April collection consisting of mussels that had already completed spawning and were recovering (Colaço et



al. 2006). These same mussels kept in aquaria for over 1 yr further maintained their spermatogenesis and spawned at the same period of the year as wild mussels (January to February 2004, Colaço et al. 2006, Kádár et al. 2006), supporting a main single period of spawning. Dixon et al. (2006) estimated a main spawning period in MG mussels from late December to January that appeared to be timed to take advantage of a winter/early spring bloom in primary production in the euphotic zone.

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