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Nutritional relations of deep-sea hydrothermal fields at the Mid-Atlantic Ridge: a stable isotope approach

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Abstract

Nutritional relations among invertebrates from the hydrothermal vent fields at the Mid Atlantic Ridge (MAR) were studied via the carbon and nitrogen stable isotope approach. A large number of specimens of different vent species from different MAR vent fields were analysed, providing a general picture of the community structure. The isotopic composition at each vent field presents the same general trend. There is an obvious dichotomy of the trophic structure, with the mussels being significantly depleted in ^{13}C and shrimps being significantly enriched in ^{13}C . MAR and Pacific vent fields present the same picture, despite a different species composition. Primary consumers are divided into main groups according to their $\delta^{13}\text{C}$ signature: > -15 (shrimps) and $< -20\text{‰}$ (mussels). Vent predators are tightly linked to one or the other group, but a mixed diet cannot be excluded. Bathyal species are top predators, making incursions into the vent fields to profit from the large biomass. Taking into account the above associations, a descriptive trophic model was elaborated. At the base of the food chain the chemolithotrophic bacteria predominate. Four trophic levels were then distinguished: primary consumers, feeding only on bacteria; mixotrophs feeding on bacteria and small invertebrates; vent predators feeding only on small invertebrates; and finally top predators that are mainly constituted by deep-sea fauna. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The discovery of dense invertebrate assemblages clustered around hydrothermal vents along Galapagos Rift at depths greater than 2500 m

(Lonsdale, 1977) brought up questions regarding the food resources of those species. Rau and Hedges (1979) and Rau (1981) were the first to suggest that the carbon and nitrogen sources for the hydrothermal vent fauna were of local origin, in contrast to deep-sea fauna deriving their food from sunken organic matter produced at the ocean's surface. Later, Jannasch (1989) and Fisher (1990) showed that the primary producers at the base of the food chain in hydrothermal vent environments are chemoautotrophic bacteria.

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These bacteria might live symbiotically within host tissues or exist as free-living cells, attached to solid substrates or suspended in the water (Van Dover and Fry, 1994).

Deep-sea hydrothermal communities are distributed in concentric rings around the vent openings. Bacterial feeders (some alvinellids and shrimps) dwell on chimney walls (Desbruyères et al., 1983; Alayse-Danet et al., 1985; Casanova et al., 1993), while organisms living in symbiotic association with primary producers (provannid gastropods, vestimentiferans, clams and mussels) colonise the intermediate area of diffuse mild venting (Belkin et al., 1986; Cavanaugh et al., 1981; Distel and Felbeck, 1987; Felbeck, 1981; Fisher et al., 1987; Stein et al., 1988), or, like the mussels at the Mid-Atlantic Ridge (MAR), colonise chimney walls (Van Dover et al., 1996; Desbruyères et al., 2001). Filter feeding organisms (serpulids, actinarians and cirripeds) settle on the external ring. At depths exceeding 2000 m, the vent ecosystem is generally rather isolated from the typical deep-sea organisms, as they do not penetrate the vent fields. At shallower depths, or where the vent medium is diluted through subsurface plumbing, this general scheme varies. Phase separation of the vent fluid then provides a less toxic environment and allows non-endemic deep-sea vent fauna such as mobile scavengers and carnivores to enter (Van Dover, 1995).

Analysing for natural stable carbon and nitrogen isotopic composition is a useful approach to unravel 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)). The $\delta^{15}\text{N}$ isotopic signature is a better indicator of the trophic level since successive trophic levels become enriched in ^{15}N by about 3.4‰ per trophic level (Minagawa and Wada, 1984). The symbiotic associations between vent fauna and chemoautotrophic bacteria lead to lower $\delta^{15}\text{N}$ values, and lower or higher $\delta^{13}\text{C}$ values than other deep-sea fauna (see Kennicutt et al., 1992; Fisher, 1995 a review).

Several stable isotope studies have been performed on MAR species in order to understand their feeding biology. These focused on the shrimp *Rimicaris exoculata* (Gebruk et al., 2000; Polz et al., 1998; Pond et al., 1997a,b, 2000; Rieley et al., 1999; Van Dover et al., 1988) and mussels (Robinson et al., 1999; Pond et al., 1998; Trask and Van Dover, 1999). At Hanging Gardens, 21°N, East Pacific Rise (EPR) (Van Dover and Fry, 1989), and at the Galapagos (Rau, 1985; Fisher et al., 1994) nutritional relations among the different species has been studied. However, at the MAR vent fields no studies on nutritional relations among species from different groups have been carried out. This work presents the first comparative study of the trophic structures of different communities from the northern part of the Mid-Atlantic Ridge using the stable isotope approach. These results are compared with similar work in the Pacific fields by Rau (1985), Fisher et al. (1994) and Van Dover and Fry (1989). Furthermore, our study of vent communities from depths ranging from 3500 to 800 m, aims to provide more information about relative contribution of chemosynthetically vs. photosynthetically derived food substrate for heterotrophs. Finally, a descriptive trophic model for the MAR vent fields is proposed.

2. Material and methods

A total of 19 species were collected from seven sites of the northern part of the Mid-Atlantic Ridge. Description of the biological and geological characteristics of these vent fields is given in Table 1.

2.1. Sampling

Samples were collected in June 1994, August 1997 and July 1998 from the submersible “Nautilite” (samples taken by grab; slurp gun), during the DIVA 1 and 2, MARVEL, FLORES and PICO cruises, and in July 1997 from the submersible “Alvin” during the MAR97 cruise. The dominant taxa (mussels, shrimps, crabs, gastropods, polychaetes) were selected for this study.

Table 1

General community description of the Mid-Atlantic Ridge deep-sea hydrothermal vent fields with the main geological characteristics

Site	Depth (m)	Latitude	T (max.)	Type of field	Chimney walls	Diffuse venting	External ring	Scavengers and carnivorous	References
Menez Gwen	800	37°51'N	281°C	4 sites. Two at the SW flank of a volcano, two at the E flank of the volcano	<i>Mirocaris fortunata</i>	<i>Bathymodiolus azoricus</i> (host endosymbionts)	<i>Chaceon affinis</i>	<i>Segonzacia mesatlantica</i> <i>Chorocaris chacei</i>	Fouquet et al. (1995) Colaço et al. (1998)
Lucky Strike	1700	37°18'N	330°C	The field consists of several sites around a lava lake. Concentration of sites at the NW, SE and NE	<i>Rimicaris exoculata</i> , <i>Bathymodiolus azoricus</i> , <i>Branchipolynoe seepensis</i> , <i>Mirocaris fortunata</i> , <i>Amathys lutzi</i>	<i>Rimicaris exoculata</i> , <i>Bathymodiolus azoricus</i> , <i>Branchipolynoe seepensis</i> , <i>Mirocaris fortunata</i> , <i>Amathys lutzi</i>	Actinaria	<i>Segonzacia mesatlantica</i> <i>Chorocaris chacei</i> <i>Phymorhynchus</i> sp.	Fouquet et al. (1995) Langmuir et al. (1997) Van Dover et al. (1996)
Rainbow	2300	36°14'N	360°C	The hydrothermal field is hosted by serpentinites and covers an area of 250 m by 60 m with several chimneys with different degrees of activity	<i>Rimicaris exoculata</i> <i>Mirocaris fortunata</i> <i>Amathys lutzi</i>	<i>Bathymodiolus</i> n. sp. (host endosymbionts) <i>Branchipolynoe seepensis</i>	Chaetopteridae polychaetes	<i>Segonzacia mesatlantica</i> <i>Chorocaris chacei</i> <i>Phymorhynchus</i> sp.	Fouquet et al. (1998) Desbruyères et al. (2001)
Broken Spur	3100	29°10'N	365°C	The vent field lies at the intersection of two sets of cross cutting fissures. There are three large high temperature venting mounds	<i>Rimicaris exoculata</i>	<i>Bathymodiolus</i> n. sp. (host endosymbionts)		<i>Segonzacia mesatlantica</i>	Murton et al. (1995)
TAG	3600	26°08'N	365°C	Black smoker massive sulphides and vent biota occur at the junction between the floor and	<i>Rimicaris exoculata</i>		Actinaria Chaetopteridae polychaetes	<i>Chorocaris chacei</i> <i>Segonzacia mesatlantica</i> <i>Phymorhynchus</i> sp.	Copley et al. (1997) Rona et al. (1993) Galkin and Moskalev (1990)

(continued on next page)

Table 1 (continued)

Site	Depth (m)	Latitude	T (max.)	Type of field	Chimney walls	Diffuse venting	External ring	Scavengers and carnivorous	References
				east wall of the rift valley					
Snake Pit	3400	23°22'N	350°C	The vent field is located on an elongated dome, which is part of an axial neovolcanic ridge in the rift valley hydrothermal area, and consists of three mounds aligned east west	<i>Rimicaris exoculata</i>	<i>Bathymodiolus puteoserpentis</i> (host endosymbionts) <i>Branchipolynoe seepensis</i>	Actinaria	<i>Phymorhynchus</i> sp. <i>Chorocaris chacei</i> <i>Alvinocaris mar-kensis</i>	Karson and Brown (1988) Fouquet et al. (1993) Segonzac (1992)
Logatchev 3000		14°45'N	353°C	The vent area has a NW–SE extension of 500 m and includes a main hydrothermal mound with several active hydrothermal sites on its top	<i>Rimicaris exoculata</i>	<i>Bathymodiolus puteoserpentis</i> . (host endosymbionts) <i>Branchipolynoe seepensis</i>		<i>Segonzacia mesatlantica</i> <i>Munidopsis</i> sp. <i>Phymorhynchus</i> sp.	Gebruk et al. (1997)

Accompanying species of invertebrates were found among mussel and basket washings (see Trask and Van Dover, 1999). The number of specimens taken for each species depended on the density and sampling effort at each hydrothermal field and site.

2.2. Analytical method

On board samples were dissected for separation of muscle whenever possible. Dissected parts or whole animals were deep-frozen (-20°C or -80°C), and crab stomachs were preserved in formalin for visual analysis of contents under a binocular microscope. In the home based laboratory samples were lyophilised and homogenised with a mortar and pestle. When carbonate was present, subsamples for $\delta^{13}\text{C}$ were acid washed. Aliquots of dry material (1 to 2 mg for $^{13}\text{C}/^{12}\text{C}$ and 5 to 8 mg for $^{15}\text{N}/^{14}\text{N}$) were weighed into decontaminated tin cups and combusted in an Elemental Analyser (Carlo Erba NA1500). The CO_2 generated during the combustion was automatically trapped in an on-line Finnigan Mat CT-NT trapping box for cryopurification before automatic injection into the mass spectrometer (Delta E, Finnigan Mat). For nitrogen isotopic ratios, the N_2 gas produced during combustion was cryogenically trapped on a vacuum line connected to the CN analyser outlet (Marguillier et al., 1997; Marguillier, 1998). N_2 was trapped in stainless steel tubes fitted with molecular sieve (zeolite 5 Å). Within 24 h after trapping, samples were manually introduced into the mass spectrometer. The normal working standard for carbon was CO_2 produced from Carrara marble. Graphite (USGS24), sucrose (IAEA-CH-6) and polyethylene foil (IAEA-CH-7) reference materials were used as standards for carbon isotopic ratio measurements. The working standard for nitrogen isotopic ratio analysis was a high-purity N_2 from a pressurised cylinder. This standard was given the value of 0‰, as for atmospheric N_2 . Four international reference materials were used: ammonium sulphate (IAEN-N-1; IAEA-N-2 and USGS25) and potassium nitrate (IAEA-NO-3). These standards followed the same analytical processes as the unknown samples. $\delta^{15}\text{N}$ values

were adjusted with the regression line constructed with the reference material results.

Carbon isotopic composition was expressed in relation to the VPDB (Vienna Pee Dee Belemnite) reference (Coplen, 1996). $\delta^{15}\text{N}$ results were expressed relative to atmospheric nitrogen.

Carbon and nitrogen isotope ratio results are expressed in the delta notation:

$$\delta X = [(R_{\text{sample}} - R_{\text{reference}})/R_{\text{reference}}] \times 10^3, \text{ in } \text{‰},$$

where X is ^{13}C or ^{15}N and $R = ^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$.

C and N analyses were performed with a Carlo Erba NA 1500 elemental analyser, with acetanilide as standard.

2.3. Statistical methods

Differences of carbon and nitrogen stable isotope signal between species were analysed by ANOVA on raw data. The Tukey HSD for unequal N (Spjøtvoll/Stoline) test was used for a balanced *post hoc* test of ANOVA. Variance homogeneity was tested by the Bartlett test, and the normal distribution was tested by the Kolmogorov–Smirnov test with the Lilliefors correction (Sokal and Rohlf, 1981).

If non-normality and heterogeneity of the variance was observed (application of Log-ANOVA did not homogenise the variances), differences between species were analysed by Kruskal–Wallis ANOVA (test H) and Mann–Whitney U tests. Kendall Tau was the correlation test used.

The degree of significance for statistical analysis was established at the 0.05 level.

Because of the variable number of specimens collected in each group, statistical analysis was not possible for all the data.

3. Results

3.1. Overall view

The entire set of results for the MAR deep-sea hydrothermal vent fields is presented in Table 2.

In Fig. 1 $\delta^{15}\text{N}$ of all taxa are plotted against corresponding $\delta^{13}\text{C}$ for each of the seven hydrothermal vent fields studied. Individual vent field

Table 2
 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the species collected in and around Mid Atlantic Ridge hydrothermal vent fields

Species	Field	$\delta^{13}\text{C}$				$\delta^{15}\text{N}$			
		Min	Max	Mean \pm SD	<i>n</i>	Min	Max	Mean \pm SD	<i>n</i>
<i>Bathymodiolus azoricus</i>	Menez Gwen (D9)	-26.37	-22.51	-24.22 \pm 1.25	8	-4.63	-1.21	-2.53 \pm 1.27	7
	Menez Gwen (pp30/pp32)	-33.71	-29.08	-31.09 \pm 3.05	38	-15.80	-8.83	-11.82 \pm 1.49	35
	Lucky Strike (Sintra/Statue of Liberty)	-25.15	-17.02	-22.31 \pm 3.07	11	-9.44	-0.53	-4.62 \pm 3.54	6
	Lucky Strike	-32.43	-27.93	-30.16 \pm 1.21	41	-13.50	-6.38	-9.49 \pm 1.66	36
<i>Bathymodiolus</i> sp.	Rainbow	-28.68	-22.74	-26.64 \pm 1.57	25	-5.532	-1.67	-3.59 \pm 1.12	17
	Broken Spur	-36.7	-35.28	-35.98 \pm 1.00	2	-10.14	-8.51	-9.32 \pm 1.15	2
<i>Bathymodiolus puteoserpentis</i>	Snake Pit	-36.91	-33.37	-34.59 \pm 1.59	4	-15.20	-10.68	-12.47 \pm 2.16	4
	Logatchev	-29.08	-14.3	-21.36 \pm 6.08	8	-3.17	+1.92	-1.20 \pm 1.81	8
<i>Branchipolynoe seepensis</i>	Lucky Strike (-20 group)	-25.94	-15.61	-22.32 \pm 3.23	8	-7.75	-0.69	-2.11 \pm 3.70	7
	Lucky Strike (-30 group)	-31.64	-27.52	-30.33 \pm 1.28	10	-9.90	-5.05	-6.94 \pm 1.52	7
	Rainbow	-28.47	-24.98	-26.41 \pm 1.83	3	-1.67	+0.10	-0.91 \pm 0.91	3
	Snake Pit			-36.42	1			-10.14	1
<i>Mirocaris fortunata</i>	Menez Gwen	-23.91	-12.07	-17.56 \pm 4.95	11	-0.004	+7.52	+3.37 \pm 3.55	7
	Lucky Strike	-20.40	-11.27	-16.27 \pm 3.01	28	-0.63	+6.518	+3.54 \pm 2.41	17
	Rainbow	-17.24	-9.43	-12.80 \pm 3.49	5	+6.74	+8.24	+7.56 \pm 0.76	3
<i>Rimicaris exoculata</i>	Lucky Strike			-11.12	1			+7.85	1
	Rainbow	-17.04	-8.29	-10.21 \pm 2.08	32	+5.76	+8.11	+6.80 \pm 0.63	19
	Broken Spur	-17.34	-10.00	-12.76 \pm 2.77	5	+5.72	+6.99	+6.42 \pm 0.54	5
	Snake Pit	-15.15	-10.70	-12.77 \pm 1.66	7	+4.72	+7.3	+6.12 \pm 0.87	7
	TAG	-15.98	-11.77	-14.28 \pm 1.87	5	+3.83	+7.02	+5.58 \pm 1.06	4
	Logatchev	-15.97	-12.25	-14.43 \pm 2.03	4	+5.87	+8.02	+6.94 \pm 1.52	2
	Lucky Strike	-16.14	-12.32	-14.55 \pm 1.22	12	+2.94	+6.46	+4.88 \pm 1.39	6
<i>Chorocaris chacei</i>	Broken Spur	-14.54	-13.96	-14.25 \pm 0.41	2			+7.59	1
	Snake Pit			-14.21	1			+8.45	1
<i>Alvinocaris markensis</i>	Menez Gwen	-17.71	-12.61	-15.16 \pm 3.61	2	+3.90	+3.91	+3.91 \pm 0.005	2
	Lucky Strike	-21.78	-10.14	-17.87 \pm 2.56	20	+2.12	+6.49	+3.98 \pm 1.45	14
	Rainbow	-16.55	-13.97	-15.30 \pm 1.29	3	+8.22	+10.25	+9.39 \pm 1.05	3
	Broken Spur	-15.70	-15.07	-15.30 \pm 0.34	3	+7.87	+9.14	+8.35 \pm 0.69	3
	TAG	-15.89	-14.03	-14.40 \pm 1.01	3	+8.41	+8.80	+8.59 \pm 0.20	3
<i>Chaceon affinis</i>	Logatchev			-16.68	1			+6.34	1
	Menez Gwen vent field			-22.24	1			+1.44	1
	Menez Gwen geographical area	-18.50	-16.85	-17.75 \pm 0.82	4	+9.60	+11.78	+10.69 \pm 1.54	2
<i>Phymorhynchus</i> sp.	Lucky Strike			-26.65	1			-6.22	1
	Rainbow	-24.36	-22.06	-23.18 \pm 1.15	5	-1.70	+2.07	+0.20 \pm 1.36	5
	Snake Pit			-28.4	1			-5.62	1
<i>Phymorhynchus moskalevi</i>	TAG	-12.76	-10.86	-11.99 \pm 0.93	4	+6.38	+7.33	+6.86 \pm 0.47	3
<i>Phymorhynchus</i> sp.	Logatchev			-25.53	1			-1.20	1

<i>Amathys lutzii</i>	Lucky Strike	-27.38	-19.61	-23.55 ± 2.93	5	-0.056	+2.11	+0.88 ± 0.92	5
	Rainbow	-20.73	-20.07	-20.40 ± 0.47	3	+4.40	+4.85	+4.66 ± 0.31	2
Actinaria	Lucky Strike			-16.83	1			+6.22	1
	TAG			-14.79 ± 0.88	2	+7.84	+8.17	+8.00 ± 0.23	2
Amphipods	Menez Gwen	-15.41	-14.18	-21.60 ± 0.30	3	+1.23	+2.50	+1.79 ± 0.62	3
<i>Lepetodrilus</i> sp.	Menez Gwen	-21.90	-19.02	-20.12 ± 1.09	3	+1.75	+3.80	+2.49 ± 1.13	3
<i>Sericosura</i> sp.	Lucky Strike	-21.01	-22.25	-24.02 ± 2.04	5	+1.82	+5.79	+4.05 ± 1.78	5
Bacterial mat	Lucky Strike	-27.27	-29.47	-29.54 ± 0.098	2	-11.70	-9.56	-10.63 ± 1.51	2
Filamentous bacteria	Lucky Strike (Eiffel Tower)	-29.61	-30.77	-34.29 ± 2.82	4	-4.28	-0.84	-3.17 ± 2.02	3
	Lucky Strike (Sintra)	-36.61		-25.15	1			-0.90	1
Gastropoda	Rainbow			-9.09	1			+6.39	1
Coiled limpet	Snake Pit			-10.12	1			+4.74	1
Free polynoidae	Logatchev			-19.32	1			+2.07	1
Munidopsis sp.	Logatchev			-18.11	1			+7.58	1
Polychaeta Chaetopteridae	Logatchev			-25.46	1			+5.50	1
Pycnogonidae Colossendeidae	Logatchev			-16.73	1			+10.76	1

plots display similar general patterns (differences exist only in the extent of depletion or enrichment in ^{13}C and ^{15}N), with the mussels and commensal worms (*Branchiopolynoe seepensis*) always having the more negative $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures, the shrimps possessing the more positive $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures, and all the other animals (crabs, gastropods, polychaete, etc) showing intermediate signals. Logatchev is the only vent field on the MAR with mussel $\delta^{15}\text{N}$ showing positive values (Fig. 1f).

3.2. Mytilids and commensal polychaetes

Mussels (*Bathymodiolus azoricus*) dominate at the shallowest vent fields (Menez Gwen and Lucky Strike) but are absent from the TAG vent field. Two mussel species were sampled along the MAR: *B. puteoserpentis* at Logatchev and Snake Pit and *B. azoricus* at Lucky Strike, Menez Gwen (Von Cosel et al., 1999). Broken Spur and Rainbow mussels are not yet assigned to a species, but both belong to the genus *Bathymodiolus*. Despite a slight tendency for gill tissues to have more negative stable isotope values than muscle tissue, no significant difference between natural isotope ratios of these tissues of mussels ($n = 15$) was detected by the Man–Whitney U test. Therefore, the results for gill and muscle tissue were pooled. At all sites, the mussels displayed a large range of isotopic composition ranging from c.a. -16‰ to c.a. $+2\text{‰}$ for $\delta^{15}\text{N}$ and from c.a. -37‰ to c.a. -14‰ for $\delta^{13}\text{C}$. On the basis of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures, and because the data are not normally distributed, it is therefore possible to distinguish two groups of mussels at Menez Gwen and Lucky Strike vent fields. The first group, hereafter called the “ -30 group” had mean $\delta^{13}\text{C}$ values ranging from -36‰ to -26‰ , and $\delta^{15}\text{N}$ values around -10‰ ; the second, hereafter called the “ -20 group” had $\delta^{13}\text{C}$ mean signatures ranging from -24‰ to -21‰ and $\delta^{15}\text{N}$ signatures around -3‰ . Those two groups are for each vent field statistically different.

Branchiopolynoe seepensis is a commensal scaleworm found in the mantle cavity of the mussels. Kruskal Wallis test showed no significant difference between $\delta^{13}\text{C}$ signature of the scaleworm and

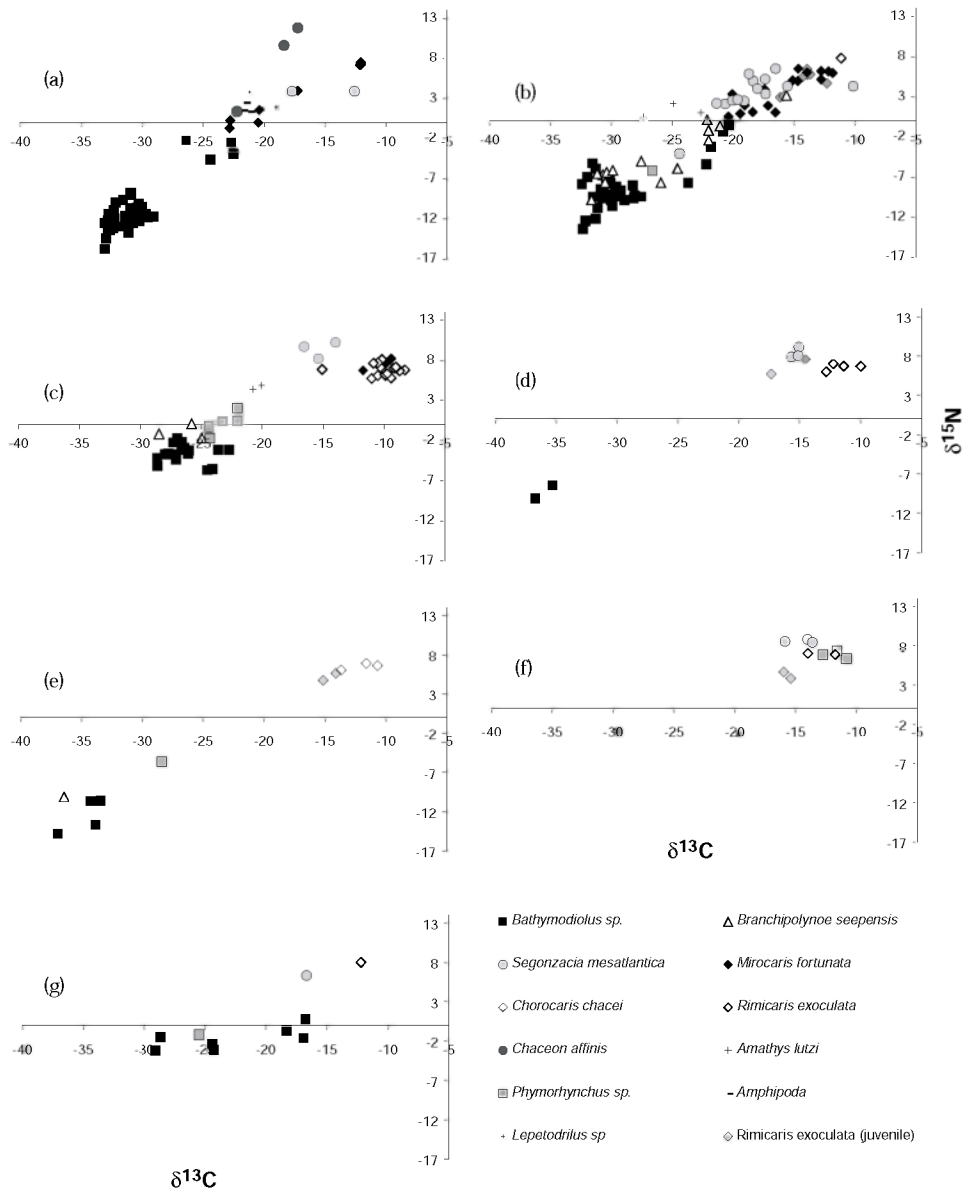


Fig. 1. Entire set of results identifying the different main groups of organisms sampled at Mid-Atlantic Ridge deep-sea hydrothermal vent fields. (a) Menez Gwen; (b) Lucky Strike; (c) Rainbow; (d) Broken Spur; (e) Snake Pit; (f) TAG; (g) Logatchev.

mussel tissue ($p = 0.25$), but indicated a significant difference for $\delta^{15}\text{N}$ ($p = 0.004$). Scaleworm tissues were enriched by c.a. 3‰ in ^{15}N relative to the host. Significant correlation values between commensals and hosts were detected for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

3.3. Caridea

Rimicaris exoculata, *Mirocaris fortunata* and *Chorocaris chacei* are the three species of bresiliid shrimps, which dominate the deepest vent fields. The *M. fortunata* and *C. chacei* are also present at

Lucky Strike and Menez Gwen, but *R. exoculata* is rare at Lucky Strike and absent at Menez Gwen. All species have isotopic signatures showing enrichment in heavy isotopes (more positive values) in comparison to mussels. *R. exoculata* is a very abundant species at all the deepest sites and is commonly observed swarming on chimney walls. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *Rimicaris exoculata* ranged from c.a. -17.4% to -8.3% and c.a. $+3\%$ to $+8.1\%$, respectively. The deepest vents (more than 3000 m deep; Broken Spur, TAG, Snake Pit and Logatchev) showed no significant difference regarding the isotopic composition of *R. exoculata*. In contrast, at the Rainbow vent field (2300 m deep) *R. exoculata* $\delta^{13}\text{C}$ values are significantly different ($p < 0.01$) from those at the other vent fields. $\delta^{15}\text{N}$ values for this species present no significant difference between vent fields.

M. fortunata $\delta^{13}\text{C}$ values range from c.a. -24% to -10% and $\delta^{15}\text{N}$ from 0% to $+8.2\%$, a depletion in ^{13}C compared to *R. exoculata*. Between vent fields, significant differences were observed in $\delta^{13}\text{C}$ but not in $\delta^{15}\text{N}$. *C. chacei* $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ranged from c.a. -16.1% to -12.3% , and c.a. $+3\%$ to $+6.5\%$, respectively, having an intermediate $\delta^{13}\text{C}$ signature compared to *R. exoculata* and *M. fortunata*. The $\delta^{15}\text{N}$ signatures are in the range of the other two species. Due to the low number of specimens sampled at Broken Spur, no statistical comparison was made. Data from the different vent fields were pooled together, and differences between the isotopic signature of *M. fortunata* and *R. exoculata* were analysed by ANOVA on raw data. The two samples are statistically different ($p < 0.0001$), with the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *M. fortunata* having a coefficient of variation much higher than *R. exoculata* (75% and 14%, respectively).

One specimen of *Alvinocaris markensis* was caught at Snake Pit. Its $\delta^{13}\text{C}$ value (-14.21%) falls within the range of values for *R. exoculata* (-12.77 ± 1.22), but its $\delta^{15}\text{N}$ shows ^{15}N enrichment of $+2.33 \pm 0.87$.

3.4. *Brachyura*

Segonzacia mesatlantica is the most common crab living within vent areas along the MAR. $\delta^{13}\text{C}$

values for this species range from -21% to -10% , and $\delta^{15}\text{N}$ values from $+0.34\%$ to $+10\%$. These values fall in the same range as the shrimps, with the $\delta^{15}\text{N}$ being heavier. Visual analyses of stomach content showed evidence of feeding on other crustaceans and small invertebrates such as polychaetes.

Chaceon affinis is a bathyal geryonid crab that forages inside the vent area at Menez Gwen. A single specimen was captured inside the vent area and two specimens outside. The specimen from the active site had a $\delta^{13}\text{C}$ value of -22.24% and a $\delta^{15}\text{N}$ of value of $+1.44\%$. The other two specimens were enriched in ^{13}C (-16.85% , -18.50%) and ^{15}N ($+11.78\%$ and $+9.60\%$). The specimen caught inside the vent area had ingested 4 individuals of *Bathymodiolus azoricus*, and the other crab's stomachs contained the remains of unidentified decapod crustaceans and fish vertebrae.

3.5. *Gastropoda* (whelk)

Gastropods belonging to the genus *Phymorhynchus* spp. were sampled from all the vent fields with the exception of Menez Gwen and Broken Spur. Their isotopic signature varies amongst vent fields, ranging from -26.75% to -10.86% for $\delta^{13}\text{C}$ and from -6.22% to $+7.33\%$ for $\delta^{15}\text{N}$. The most ^{13}C and ^{15}N depleted signatures were observed for whelks associated with mussel beds. The most enriched ^{13}C and ^{15}N signatures were measured in specimens from the field where mussels were absent and shrimps predominated (TAG hydrothermal vent field). Differences between TAG whelk signatures and the other vent fields were significant ($p < 0.05$).

3.6. *Other invertebrates*

A number of species (ampharetid, scale-worms, actinarian, limpets, and pycnogonida) were sampled from washings of dominant species and sulphide rubble. Some of them (e.g. chaetopterid), originating from the periphery of vents, were also sampled in sediments. The invertebrates studied present a wide variety of signals. Species such as the polychaete *Amathys lutzi*, a chaetopteridae

polychaete species, the limpet *Lepetodrilus* sp., the amphipods and the pycnogonid *Sericosura* sp., had a wide range of $\delta^{13}\text{C}$ values, from -27‰ to -19‰ , while the $\delta^{15}\text{N}$ values ranged from $+0.88\text{‰}$ to $+4\text{‰}$. In contrast, the gastropod collected at the Rainbow vent field and the Snake Pit coiled limpet showed a clear ^{13}C enrichment ($\delta^{13}\text{C}$ values are -9‰ and -10‰ , respectively) compared to *Lepetodrilus* sp., but only slightly enriched $^{15}\text{N}/^{14}\text{N}$ compositions ($\delta^{15}\text{N}$ of $+6\text{‰}$ and $+4\text{‰}$, respectively).

Animals like actinarian, galatheid squat lobster *Munidopsis* sp. and the pycnogonid Colossoendeidae, collected at the periphery or outside the vent fields, had a $\delta^{13}\text{C}$ signature ranging between -18‰ and -14‰ and a very positive $\delta^{15}\text{N}$ signature ranging from $+6\text{‰}$ to $+10\text{‰}$.

3.7. Bacterial mats

Bacterial mats are abundant on all mineral and biogenic substrates. They were sampled and analysed from two Lucky Strike vent sites. Filamentous bacteria from one site had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of -34‰ and -3‰ , respectively, and those from another site had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of -25‰ and -1‰ , respectively.

Furthermore, a non-filamentous bacterial mat collected at Lucky Strike had an average $\delta^{13}\text{C}$ signature of -29‰ and a much lighter $\delta^{15}\text{N}$ signature of -10‰ .

4. Discussion

The major feature observed at all the vent fields is that the shape of the $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ plot (Fig. 1) is a diagonal pattern showing very negative $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values at one end (mussels and commensal worm) and less negative $\delta^{13}\text{C}$ signals and positive $\delta^{15}\text{N}$ signals (shrimps) at the other end. This dichotomy is present at all the vent fields, independent of the variability existing inside the vent fields, with the exception of the TAG vent field, where no mussels are present. Isotopic signatures of all other animals are in between mussels and shrimps. No depth pattern has been observed between the shallow site (Menez Gwen)

and all the deeper sites, with the isotopic signatures being dependent more on chemoeological characteristics. The phase separation of fluids, and the depletion in metals of the shallowest sites, will allow the common deep-sea bathyal fauna to make incursions to the hydrothermal vent fields to feed, as in the case of the geryonid crab *C. affinis*.

4.1. Mussels and commensal polychaete

It has been well documented that the MAR mussels host both thio- and methanotrophic symbionts (Fiala-Médioni and Le Pennec, 1987; Cavanaugh et al., 1992; Distel et al., 1995; Fiala-Médioni et al., 1996; Robinson et al., 1999; Trask and Van Dover, 1999). It is then expected that mussels exhibit ^{13}C depletion due to the chemosynthetically derived organic carbon (Ruby et al., 1987). The lowest $\delta^{13}\text{C}$ values (-30 group) of the mussels at MAR overlap with those observed for different species of mussels from Pacific hydrothermal vents, known to host thiotrophic symbionts (Rau and Hedges, 1979; Rau, 1985; Ruby et al., 1987; Fisher et al., 1988; Van Dover and Fry, 1989; Kim and Sakai, 1991; Fisher et al., 1994; Shanks et al., 1995; Dubilier et al., 1998). However, the Menez Gwen and Lucky Strike hydrothermal fields also host mussels with different isotopic characteristics; these were identified as the “ -20 ” group. More interesting, this -20 group consists of specimens from particular sites inside the vent field. At Lucky Strike, Trask and Van Dover (1999) also found two groups of mussels with distinct $\delta^{13}\text{C}$ signatures (“ -20 ” and “ -30 ” groups) and, moreover, the different $\delta^{13}\text{C}$ signatures were from mussels belonging to the same sites as in this study. Trask and Van Dover (1999) showed differences in the proportion of methanotrophic and thiotrophic endosymbionts in mussels and hypothesised that the difference was due to a dependency on different symbionts. The -20 group would depend more on methanotrophic bacteria, and the -30 group more on thiotrophic bacteria.

It can be seen from Table 2 that the two samples of filamentous bacteria from two different sites at Lucky Strike present the same difference between

$\delta^{13}\text{C}$ values as observed for the mussels sampled at the same sites. Assuming that the mussels rely essentially on symbiotic bacteria and are of the same species at both Lucky Strike sites, the similar isotope signatures suggest that the filamentous bacteria and the symbiotic bacteria use the same source of dissolved inorganic carbon (DIC). Previous data for $\delta^{13}\text{C}_{\text{DIC}}$ point to differences from site to site at Lucky Strike (Colaço et al., 1999). Von Damm et al. (1998) identified two distinct source waters at Lucky Strike based on the element composition of the site vent fluids inside the vent field. When the characteristics of these sites with the different $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of mussel data are compared, the same two groups are obtained, reinforcing the hypothesis that the fluid source plays an important role on the $\delta^{13}\text{C}$ mussel signature. The presence of both types of symbiont, the large variability of isotopic discrimination amongst methanotrophic bacteria (Jahnke et al., 1999), and the Trask and Van Dover (1999) hypothesis call for more enzymatic and metabolic studies of vent mussels to explain the existence of two groups inside a single vent field, especially given the fact that those two groups are not found only within the Lucky Strike hydrothermal vent field, but also on the Menez Gwen vent field.

We also observed differences in the $\delta^{15}\text{N}$ signatures between the two groups of mussels, with the more negative values (-10‰ vs. -3‰) present in the -30 group. Different types of nitrogen source, or different types of bacterial metabolism (methanotrophic or thiotrophic), can be a possible explanation for those differences.

The generally very low $\delta^{15}\text{N}$ values for the mussels suggest that their nitrogen source is of local origin (e.g., through their symbiosis with autotrophic bacteria, Van Dover and Fry, 1989). According to Lee and Childress (1994, 1995), mussel symbionts assimilate essentially ammonium and, to a lesser extent, nitrate. The high concentrations of ammonium in hydrothermal solutions (Sarradin et al., 1998, 1999) are likely to preclude conditions of nutrient limitation for the autotrophs. According to Waser et al. (1998) different primary enzymes for NH_4^+ and NO_3^- assimilation discriminate up to 20‰. In this situation the low

$\delta^{15}\text{N}$ values could result from maximal depletion of the heaviest isotope by enzymatic discrimination in a condition of nutrient substrate excess.

Throughout this study we observed that the $\delta^{13}\text{C}$ isotopic composition of the commensal polychaete *B. seepensis* is very similar to that of its host (the mussel). This result is in agreement with previous studies (Peterson and Fry, 1987; Minagawa and Wada, 1984; Desbruyères et al., 1985; Fisher et al., 1988, 1994). It presents the same $\delta^{13}\text{C}$ signature but a higher $\delta^{15}\text{N}$ signature, reflecting one increment in the food chain.

4.2. Shrimps

Vent shrimps (adults) are usually known as bacterial grazers, epibiotic bearing (*Rimicaris exoculata*) (Segonzac et al., 1993; Van Dover et al., 1998; Polz et al., 1998; Gebruk et al., 2000; Pond et al., 2000), scavengers or detritus feeders (*M. fortunata*), and scavengers or predators (*Alvinocaris markensis* and *C. chacei*) (Segonzac et al., 1993; Gebruk, 1996; Gebruk et al., 2000).

Several studies suggest that *R. exoculata* subsists largely on a bacterial diet (e.g. Polz et al., 1998; Rieley et al., 1999; Segonzac et al., 1993; Van Dover et al., 1988; Gebruk et al., 2000; Pond et al., 2000). Our data indicate that if this is the case these bacteria should have significant ^{13}C enrichments compared to the symbiotic bacteria associated with the mussels and to the free-living bacteria. Robinson and Cavanaugh (1995) showed that bacteria might use different Rubisco (Ribulose biphosphate carboxylase oxygenase) forms. This carboxylating enzyme has two forms, I and II, discriminating differently against ^{13}C . Form I discriminates in the order of 29‰ (Goericke et al., 1994), and form II discriminates less (17.8‰; Roeske and O'Leary, 1984, 1985). Robinson et al. (1998) cloned and sequenced the gene coding for Rubisco II in the chemoautotrophic symbiont of *Riftia pachyptila*, a Pacific deep-sea vent vestimentiferan tubeworm that presents a $\delta^{13}\text{C}$ signature close to -11‰ (Desbruyères et al., 1983; Van Dover and Fry, 1989). Similarly high $\delta^{13}\text{C}$ values are observed for shrimps at MAR vent fields. Paoli and Tabita

(1998) showed that Rubisco form II requires more CO₂ and less O₂. These conditions would be present at warmer environments where shrimps thrive (Childress et al., 1993; Sarradin et al., 1998). Likewise, for the Pacific vents, animals (alvinellidae and vestimentiferan) that live in a warmer environment have $\delta^{13}\text{C}$ signals close to -11% (Desbruyères et al., 1983; Van Dover and Fry, 1989; Fisher et al., 1994).

In general, the shrimp isotopic values reported by others (see Van Dover et al., 1988, 1996; Polz et al., 1998) for the same vent fields and the same specimens fall within the range of values observed in our study. Of all groups, shrimps were the most enriched in ^{13}C ($\delta^{13}\text{C}$ between -20% and -9%). On average such signals are less negative than expected if detritus of phytoplankton origin represented a significant fraction of their food (expected to have $\delta^{13}\text{C}$ values close to -19% , -22% ; e.g. Bentaleb, 1994; Fontugne and Duplessy, 1981). Furthermore, the possibility that adult shrimps could subsist, to a significant degree, on phytoplankton detritus settling from the surface waters can be dismissed on the basis of the carbon isotopic composition of the material recovered with sediment traps at Rainbow field (Khrifounoff et al., 2001). $\delta^{13}\text{C}$ values for particles collected near the smokers have $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of -20% and $+1.8\%$, respectively, while the sediment trap deployed outside the influence of the vent field (as pelagic reference) had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of -24% and $+4\%$, respectively. In comparison, Rainbow shrimps have values between -16% and -9% indicating a carbon source significantly more enriched in ^{13}C and depleted in ^{15}N than surface ocean particles and closer to the vent particles. This excludes phytoplankton as a substantial part of their diet.

For *Mirocaris fortunata*, a large variability of isotopic signature for specimens from the same site is observed (e.g. Lucky Strike, Fig. 1). Although shrimps are potential consumers of (dead) mussel tissue, the isotopic distance to the mussels is too large, greatly exceeding the 3.4% (for $\delta^{15}\text{N}$) and 1% (for $\delta^{13}\text{C}$) differences generally accepted for successive trophic levels (Minagawa and Wada, 1984). Thus it is likely that shrimps are feeding mainly on another food source, of which che-

moautotrophic bacteria are a good candidate (Segonzac et al., 1993; Polz et al., 1998; Pond et al., 1997a, b; Van Dover et al., 1996, 1988). The presence of small invertebrates in some *M. fortunata* stomachs indicates that they can also be predators, scavengers or detritivores and emphasises further their opportunistic feeding behaviour. Finally, the enrichment in ^{13}C and ^{15}N of *C. chacei* relative to *M. fortunata* (Fig. 1) and other invertebrates suggests *C. chacei* prey on the latter. Mussels and commensal worms can be excluded as food sources, because of the large $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ distance.

Alvinocaris markensis enrichment in $\delta^{15}\text{N}$ and the same $\delta^{13}\text{C}$ signature of shrimps indicates that this species is consuming the other shrimps, belonging then to a higher trophic level.

4.3. Crabs

The isotopic values found for the crab *Segonzacia mesatlantica* largely overlap with those observed for shrimps and mussels, with a slight tendency to approach the ^{13}C enriched values for shrimps. Visual analysis of stomach contents revealed the presence of bresiliid shrimps and other small invertebrates (such as unidentified polychaetes) but not of mussels. Rau (1985) also observed in the Galapagos crabs a $\delta^{13}\text{C}$ values closer to vestimentifera (-11%) than to bivalves (-30% to -37%). However, such as observed for the Galapagos the final isotopic composition of the crab is set by the relative contributions of mussels and shrimps plus other invertebrates as food sources. Overall these observations suggest that mussels are not a very important prey for *S. mesatlantica*.

The deep-sea crab *Chaceon affinis* is common at bathyal depths and is exploited commercially at Madeira and the Azores archipelago. This crab takes advantage of the hydrothermal mussels in the Menez Gwen field, as suggested by its stomach content analysis. Specimens caught inside the vent field are lighter in ^{13}C and ^{15}N , whereas specimens caught outside the vent field are heavier in ^{13}C and ^{15}N , reflecting directly the dependency on hydrothermal or pelagic food sources.

4.4. *Whelk*

The bathyal gastropod *Phymorhynchus* spp. is always found at hydrothermal fields. It is a necrophage, and with the exception of the TAG site it is always associated (isotopically) with the mussels rather than with the shrimps. Because of the absence of mussels at TAG, shrimps, along with other small invertebrates, are the main source of food for this gastropod. This is deduced from their higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values at TAG compared to *Phymorhynchus* spp. values at vent fields where mussels are present. At the Galapagos vent field Fisher et al. (1988, 1994) also observed this tight link, in particular for mussels at the peripheral sites. The genus *Phymorhynchus* spp. is also known from the non-hydrothermal deep-sea environment. The link observed here with the vent mussel is probably determined by the fact that the mussels' normal niche is at colder environments than that of hydrothermal shrimps (Sarradin et al., 1999). The tight link in TAG is probably due to the scavenger behaviour of this species, feeding on dead shrimps, as they fall from warmer to colder environments.

4.5. *Other invertebrates*

The distribution of stable isotope ratios for the other invertebrates suggests three groups of distinct trophic levels. The first group, with negative $\delta^{13}\text{C}$ values and positive $\delta^{15}\text{N}$ values, seems to feed on several food sources, from which bacteria are a good candidate although detritivore behaviour cannot be excluded. The second group, with fairly high $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values presents a signal similar to the one found for the shrimp *R. exoculata*. This suggests that these gastropods and *R. exoculata* depend on the same type of bacteria. The third group had values similar to those observed for the crab *C. affinis* caught outside the vent field, indicating that they occupy a high trophic level and profit occasionally from the vent communities, but not exclusively.

4.6. *Trophic structure*

MAR and Pacific vent fields present a similar general trophic organization. Primary consumers

are divided in two main groups according to their $\delta^{13}\text{C}$ signature: $> -15\text{‰}$ and $< -20\text{‰}$. The first group corresponds to organisms that live in a warmer environment than the second one. Vent predators are tightly linked to one or to the other group, but a mixed diet can not be ruled out as suggested by the carbon signal. The first group of primary consumers ($> -20\text{‰}$) corresponds to the mussels and small invertebrates like amphipods, *Amathys lutzi*, *Lepetodrilus* sp. and *Sericosura* sp. The second group ($< -15\text{‰}$) comprises shrimps and gastropods.

From this study and previously reported $\delta^{13}\text{C}$ signatures of MAR hydrothermal mussels and shrimps (see Pond et al., 1998, 2000; Robinson et al., 1999; Trask and Van Dover, 1999; Gebruk et al., 1997; Polz et al., 1998; Segonzac et al., 1993) we can suggest a descriptive trophic model (Fig. 2) for the MAR vent fields, in which chemolithotrophic bacteria (symbiotic and free-living) form the basis of the food chain independently of their metabolic characteristics (see Karl, 1995). The primary consumers are (1) mussels, which host these symbiotic bacteria, (2) shrimps (*R. exoculata*), which feed on free-living bacteria in the warmer environments, and (3) small invertebrates that feed on bacteria or are detritivores and live between the intricate and dense byssus net that the mussels colonies develop. The crab *Segonzacia mesatlantica* has a larger predation impact on the shrimps and the small invertebrates, whereas the gastropod *Phymorhynchus* sp. relies almost exclusively on the mussels. The shrimps *C. chacei* and *M. fortunata* can be considered mixotrophic, feeding on bacteria and other shrimps or small invertebrates.

Finally, as top predators, we find different bathyal species making incursions into the vent fields to feed on this deep-sea oasis, which offers a larger source of food as compared to the deep-sea environment. The deep-sea crab *C. affinis* and, according to Saldanha (1994), bathyal fishes that live close to these vents, belong to this trophic level.

In a changing environment like the hydrothermal vents (Hessler et al., 1985, 1988; Fustec et al., 1987), where the environmental conditions are extreme, just a few species can survive. The endemism ratio is quite high, and the species richness low. Just a few predators are able to adapt

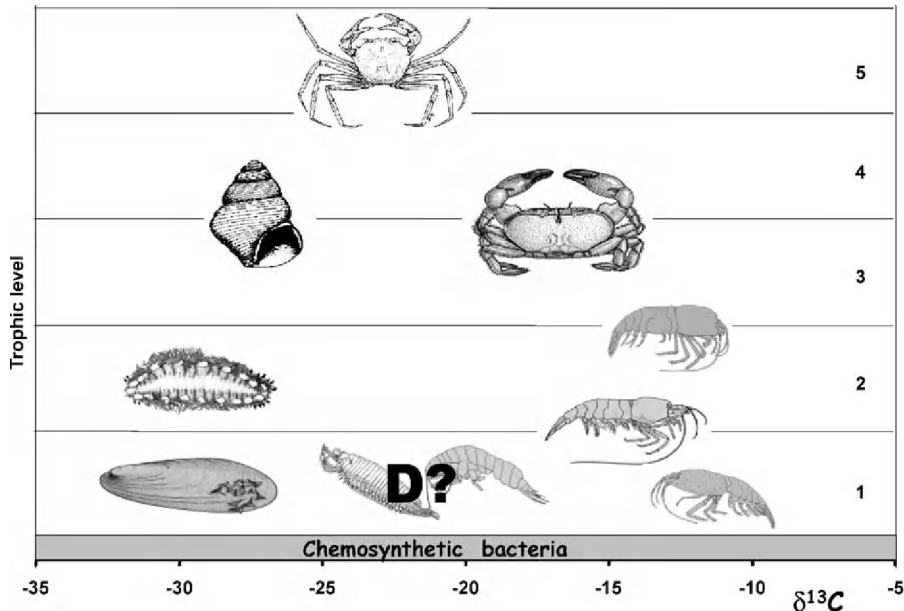


Fig. 2. Suggested trophic model of Mid-Atlantic Ridge hydrothermal communities, showing the trophic structure dichotomy. Animals are represented according to their trophic level and the most frequent $\delta^{13}\text{C}$ isotopic signatures. D? refers to uncertain trophic position. These invertebrates might be primary consumers or detritivores. The chemosynthetic bacteria are the base of the hydrothermal vent food chains (Karl, 1995).

to these conditions. These circumstances would favour a community with a short food chain having just a few trophic levels. The similarity of trophic structure at the different MAR fields reflects the absence of specific predation by normal bathyal deep-sea organisms on these communities, and also a low diversity.

The stable isotope approach can be a useful tool to reveal trophic links, since it may reveal unexpected trophic dependencies. However, stable isotopes are not the tool to study processes at a single trophic or guild level, for which enzymatic, genetic, and biochemical studies are probably more appropriate.

The nutritional relations of the different MAR vent fields are very similar with two main groups of primary consumers and predators depending on one, or both groups of primary consumers.

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