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Bioaccumulation of Hg, Cu, and Zn in the Azores Triple Junction hydrothermal vent field food chains

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Abstract: In this work, mercury (Hg), copper (Cu) and zinc (Zn) concentrations and tissue distribution are determined in seven benthic invertebrates species (the key species) from the Mid Atlantic Ridge (MAR) hydrothermal vent fields. The samples were collected from three hydrothermal vent fields – Menez Gwen, 840 m; Lucky Strike, 1700 m and Rainbow, 2300 m – near the Azores Triple Junction. These fields are characterized by different depths, geological context and chemical composition of the hydrothermal fluid, particularly the metal content, which is reflected by the metal concentrations in the organisms. Indeed, our results show that organisms from Menez Gwen presented the highest Hg concentrations, while those from Lucky Strike and Rainbow were richer in Cu and Zn. The potential transfer of these metals through two trophic links are also evaluated and include (1) the mussel *Bathymodiolus azoricus* and the commensal worm *Branchipolynoe seepensis*, and (2) three different species of shrimps and the crab *Segonzacia mesatlantica*. No evidence of Hg biomagnifications in either of the vent food chains is clearly observed but an increase in Hg accumulation from prey to predator in the crustacean food chain. The same pattern was observed for Cu and Zn, even though these metals are not known to be generally biomagnified in food chains.

Keywords: heavy metals; trace elements; bioamplification; trophic transfer; trophic relations; Mid-Atlantic Ridge

1. Introduction

Deep-sea hydrothermal vent communities develop in the interfacial zone where hydrothermal vent fluids and seawater mix. This zone is characterized by its instability, leading to continually fluctuating environments. Thus, vent organisms are constantly switching from an environment enriched in the reduced chemical compounds from vent fluids to cold oxygenated seawater. The fluids are especially rich in sulphides, methane and heavy metals, which are potentially toxic for the exposed organisms (Fisher, 1990; Childress and Fisher, 1992).

The three hydrothermal vent fields, i.e. Rainbow, Menez Gwen and Lucky Strike, located on the Mid Atlantic Ridge (MAR), near the Azores Triple Junction (ATJ), are characterized by their different end-member fluid chemical compositions, depths, geological contexts and associated biological communities (Desbruyères et al., 2000, 2001).

The hydrothermal fluid emitted at Rainbow displays by far the highest metal concentrations, while the fluids collected in the Menez Gwen site display the lowest. The variation in fluid composition is related to phase separation processes (boiling/distillation of sub-surface vent fluids) and to the nature of the basement rock (basaltic and/or ultramafic) (Charlou et al., 2000, 2002; Desbruyères et al., 2000; Douville et al., 2002). In these regions, the presence of two food chains in the prevailing food web have been highlighted using stable isotopes (Colaço et al., 2002) and include (1) the mussel *Bathymodiolus azoricus*, the commensal worm *Branchiopolynoe seepensis* and the whelk *Phymorhynchus* sp., and (2) different species of shrimps and the crab *Segonzacia mesatlantica*.

Mercury (Hg) is a non-essential metal that can be found in all surface media. It is the only metal that consistently biomagnifies through the food chain, i.e., predators accumulate higher tissue amounts than their food contains (Monteiro et al., 1996). High temperatures found in the Earth's mantle zones induce high Hg mobility, leading to its continuous diffusion into the lithosphere surface. Diffusion into the lithosphere may be huge as in the case of the New-Zealand hydrothermal vents, where sediments contain pure Hg and Hg sulphides as a consequence of release from vent fluids (Stoffers et al., 1999). The only Hg data on the vent fluids from the MAR available indicates that the concentrations are very low; most of the times below the detection limit of commonly used analytical techniques (Kadar et al., 2005).

Copper (Cu) and zinc (Zn) are essential metals vital to enzymes and respiratory pigments (White and Rainbow, 1985). Hydrothermal fluids are rich in Cu and Zn in comparison with the background seawater. Transfer of metals through food chains has been considered in studies dating back to the 1960s (Bryan, 1964; Hoss, 1964). It is now considered that the dietary exposure is the major route for metal bioaccumulation for many marine animals (Wang, 2002).

The objective of this study is to determine the behaviour of Hg, Cu and Zn along the hydrothermal vent food chains of the MAR. Tissue concentrations and distribution of the three metals were determined for the key invertebrate species from the three Azores hydrothermal vents fields (Menez Gwen, Lucky Strike and Rainbow) in order to investigate the transfer of metals between prey and predator.

2. Materials and methods

The study region comprises the ATJ hydrothermal vents with Menez Gwen (37° 50 N) at 840 m; Lucky Strike (37° N) at 1700 m and Rainbow (36° N) at 2300 m.

A general description of the three vent fields (Rainbow, Lucky Strike and Menez Gwen) and associated biological communities can be found in Desbruyères et al., 2000, 2001. The main characteristics of end-member vent fluids at Menez Gwen, Lucky Strike and Rainbow are presented in Table 1.

2.1. Sample collection and preparation

The French ROV Victor 6000 collected faunal samples during the ATOS cruise (2001, RV L'Atalante). Organisms were collected either with the slurp gun or with the packman arm of the ROV. Table 2 summarises the characteristics of the sampled animals. Bacteria were collected from hard surfaces. Before dissection, all specimens were cleaned with a synthetic fibre brush in order to remove particles that could contaminate the samples. Then, accurate dissections of the animals were performed on board and tissue samples were deep frozen in individual microtubes.

Stomachs and intestinal tracts were separated for scanning electron microscope (SEM) analysis and energy dispersive X-ray analysis (EDAX). Back at the laboratory, the separated tissue samples were freeze-dried then homogenised for heavy metal analysis.

2.2. Analytical methodology

Determination of total Hg

Samples were digested in a microwave unit (Unicam microwave) using trace metal grade with negligible Hg content nitric acid 60% in a closed fluorocarbon container. Each sample was digested under pressure in order to ensure the complete destruction of organic compounds containing the metal. Total Hg was determined by atomic fluorescence spectrometry using a 7474 method with a PS Analytical® Millennium Merlin Fluorescence Detector.

Accuracy of the method was monitored throughout the study with standards of inorganic Hg and reference materials, i.e., Plankton CRM414 n°137 ($0.276 \pm 0.18 \mu\text{g Hg.g}^{-1}$) measured ($0.309 \pm 0.2 \mu\text{g Hg.g}^{-1}$) and Mussel Tissue CRM 278R n°276/7 ($0.196 \pm 0.009 \mu\text{g Hg.g}^{-1}$), measured ($0.180 \pm 0.062 \mu\text{g Hg.g}^{-1}$).

Determination of total Cu and Zn

Each digested sample was analysed for Cu and Zn using a flame atomic absorption spectrophotometer Varian 250 Plus with deuterium background correction. Accuracy of the method was monitored throughout the study with standards of inorganic Cu and Zn and reference materials DOLT-2 NRCC ($25.8 \pm 1.1 \mu\text{g Cu.g}^{-1}$ and $85.8 \pm 2.5 \text{Zn.g}^{-1}$), measured ($26.6 \pm 0.5 \mu\text{g Cu.g}^{-1}$ and $87.6 \pm 1.0 \mu\text{g Zn.g}^{-1}$).

Interference and sensitivity due to matrix and pre-treatment were assessed by the method of standard additions. Recoveries of added inorganic metals averaged 95–103% ($n = 9$ for Hg and $n = 5$ for Cu and Zn).

Metal concentrations are given in micrograms per gram on a dry weight basis ($\mu\text{g.g}^{-1}$ dwt). Detection limits ($\mu\text{g.g}^{-1}$ dwt), calculated as three SD of eight blanks were 0.5 for Cu, 3 for Zn and 0.005 for Hg.

To determine the raw composition of the minerals present in the stomachs and intestinal tracts, a Philips XL30 scanning electron microscope (SEM) equipped with an EDAX detector was used at 15 kV.

2.3. Data analysis

Statistical comparison of metal concentrations among vent fields and among species was done using the non-parametric Kruskal–Wallis test using StatSoft Software. This test can deal with data either not normally distributed, with significantly different variance or with a low n. The significance level was set at $\alpha = 0.05$.

3. Results

3.1. Bioaccumulated concentrations and comparison among sites

Table 3 shows the Hg, Cu, and Zn concentrations in the tissues of the different species from the three vent fields. For the same species occurring at different vent fields like the mussel and the hydrothermal crab, invertebrates from Menez Gwen generally presented higher Hg concentrations, whereas individuals from Lucky Strike and Rainbow were richer in Cu and Zn.

From the three vent fields the muscle almost generally presented the lowest Hg concentrations (ranging from 0.80 for mussels from Menez Gwen to 0.01 $\mu\text{g}\cdot\text{g}^{-1}$ dwt for the juvenile shrimp *Rimicaris* from Rainbow). Generally the digestive gland presents the higher Hg concentrations, with the exception of the mussel gills at the Lucky Strike vent field, which displayed a clearly higher value.

Cu was also higher in the digestive gland than in the muscle tissue or in the mussel mantle in all the vent fields. In the former tissue, the metal concentrations ranged from 18 $\mu\text{g}\cdot\text{g}^{-1}$ dwt in the mussel from Rainbow to 2850 $\mu\text{g}\cdot\text{g}^{-1}$ dwt in the hydrothermal crab at Rainbow. In the later tissue, Cu concentrations ranged from 4 $\mu\text{g}\cdot\text{g}^{-1}$ dwt in the Rainbow mussel to 125 $\mu\text{g}\cdot\text{g}^{-1}$ dwt in the hydrothermal vent crab muscle from Rainbow and Lucky Strike (Table 3).

In the first food link composed of the mussel and the commensal worm, there was a significant difference in metal concentrations in the tissues of mussels from the different hydrothermal vent fields (Table 3). The mussel tissues themselves also varied in Hg, Cu and Zn content with the gills and digestive gland presenting the highest concentrations. The commensal worm (whole tissues), absent at Menez Gwen, displayed significantly higher Hg concentrations at Lucky Strike than at Rainbow, whereas Cu and Zn concentrations were not statistically different. In the second food link (shrimps,

hydrothermal crab, deep-sea crab), crustaceans from the different vent fields showed a similar pattern of metal concentrations in the muscle tissues. From the hydrothermal species, the endemic crab, *S. mesatlantica*, displayed not only the highest Hg concentrations at Menez Gwen but also the lowest Cu concentrations. Interestingly, Zn concentrations in muscle tissue were similar at all vent fields for this crab species, suggesting a regulation capacity of this metal. The abdomen muscle of the different shrimp species presented the overall lowest values of Hg, Cu and Zn. The Hg concentrations in the abdominal muscle of *M. fortunata* were lower at Menez Gwen than at Rainbow. Muscular Cu concentrations in *R. exoculata* were similar for adults and for juveniles (Table 3). However juveniles showed lower Hg and higher Zn concentrations than adults (0.01 and 0.17 $\mu\text{g}\cdot\text{g}^{-1}$ dwt for Hg and 228 and 58 $\mu\text{g}\cdot\text{g}^{-1}$ dwt for Zn, respectively). The deep-sea crab *Chaceon affinis*, considered a top predator, showed muscle concentrations of 0.77, 33 and 221 $\mu\text{g}\cdot\text{g}^{-1}$ dwt for Hg, Cu, and Zn, respectively. The Cu and Zn values were lower than those recorded for the hydrothermal crab, while the Hg was higher.

The digestive glands of *C. chacei* and *C. affinis* also contained remarkably high Cu concentrations (264 and 454 $\mu\text{g}\cdot\text{g}^{-1}$ dwt, respectively).

Cu concentrations were found to be particularly high in the digestive gland of the hydrothermal vent crab *S. mesatlantica*. However, specimens from Menez Gwen showed the lowest Cu concentrations but the highest Hg and Zn concentrations. The deep-sea crab presented lower Hg and Zn values for the digestive gland than the hydrothermal crab for the same vent field, while the Cu concentrations were higher for the deep-sea crab.

3.2. Stomach and digestive track contents

Table 4 presents the results of SEM and EDAX observed minerals in different states and with different compositions inside the stomachs of the four groups analysed. The species examined presented hydrothermal minerals in their stomachs, either as anhydrite solutes, which are typically found at the vent plume and start to dissolve at temperatures below 150 °C, or as minerals from chimney erosion, like pyrrhotite, pyrite, and chalcopyrite that have been formed at high temperatures. No relation was found between the animal distribution and the temperature of mineral formation, or the state of

oxidation. All the analysed stomachs presented very high concentrations of Hg attaining $5.3 \mu\text{g}\cdot\text{g}^{-1}$ dwt in the shrimp from Lucky Strike (see Table 5).

4. Discussion

4.1. Bioaccumulation and distribution of metals in the organisms

Two types of factors affect metal bioavailability for aquatic biota: physico-chemical factors acting outside the organisms, and biological factors acting within the organisms. The former will affect all biota in almost the same way depending on the characteristics of the environment (Borgmann, 2000) whereas the biological factors may act in different ways. Among the latter, the biological uptake from ingested food is probably the most important (Borgmann, 2000). This defines the assimilation efficiencies of the contaminants, which are critical for understanding their bioaccumulation and trophic transfer in aquatic invertebrates (Wang and Fisher, 1999).

The higher Hg concentrations recorded for the animals from Menez Gwen are probably related to the fluids and surrounding environment. Indeed, oceanic ridges diffuse Hg from their spreading centre. Analyses at Famous area (MAR) showed Hg concentrations in seawater reaching $7.8 \cdot 10^{-3} \mu\text{M}$ (Carr et al., 1974). Since Hg is more concentrated in gas bubbles than in spring water, it may be enriched in sediments because of vapour phase migration (Stoffers et al., 1999). Therefore, the generally higher Hg concentrations found in organisms from the shallowest vent – Menez Gwen – are not surprising as the vent fluid emitted corresponds to the light fraction enriched in gases after the phase separation process. Furthermore, this had already been observed comparing total Hg concentrations in *B. Azoricus* (whole animals) from Menez Gwen, Lucky Strike and Rainbow (Martins et al., 2001). The Menez Gwen vent field is also depleted in essential metals (Cu and Zn) due to these phase separation fluids, whereas the deeper vent fields, Lucky Strike and Rainbow, are enriched in these metals. Thus, it is not surprising that similar Cu and Zn concentrations were found at these two sites, while concentrations in the same species from Menez Gwen were lower (Table 3). However, the gradient of Cu and Zn concentration encountered in the end member fluid (Rb>>LS>MG, cf. Table 1) is not observed when comparing metal concentration in the organisms. This highlights the necessity to know the metal concentration in the environment surrounding the studied organisms.

Comparing Rouse et al. (1998) *B. azoricus* metal concentrations from Lucky Strike and Menez Gwen to the present study, their values fell within the same range for Lucky Strike with the exception of a slight increase in Cu in the mussel gills, while those reported for Menez Gwen were lower. As the environment at Menez Gwen is known to be heterogeneous (Colaço unpublished data), it may be that the samples from the previous work came from an environment influenced by a fluid with different properties. This suggests the occurrence of time-related variations. Hence, there is a need to monitor metal concentrations in organisms which could give an indication of variation of the fluid composition.

In the deepest environment (Lucky Strike and Rainbow), the crab *S. mesatlantica* presented very high Cu concentrations in the digestive gland (reaching as high as 3000 $\mu\text{g}\cdot\text{g}^{-1}$ dwt (47 μM)) in relation with corresponding end member fluid concentrations, 141 μM and 39 μM , respectively. Hydrothermal crustacean haemolymph exhibits ionic composition similar to that of the surrounding environment, with the exception of Cu, Ca and Mg (Chausson, 2001). Very high concentrations of Cu in the digestive gland of the crab *Bythograea thermydon* from the EPR-13°N have also been reported (Cosson and Vivier, 1997). These authors linked up these high levels to haemocyanin synthesis and to the accumulation of Cu-rich granules associated to sulphur in special cells. Indeed, decapod crustaceans are known to accumulate Cu in their digestive gland, allowing them to maintain constant body Cu concentrations under varying external dissolved Cu levels. From a threshold of dissolved Cu concentration, the metal starts to be accumulated (White and Rainbow, 1982, 1985). The storage of Cu in the digestive gland allows the synthesis of the respiratory pigment, haemocyanin. This mechanism is also known in cephalopods, which digestive gland can exhibit extremely high Cu concentrations i.e. 15000 $\mu\text{g}\cdot\text{g}^{-1}$ dwt (Martin and Flegal, 1975). This suggests the presence of regulatory mechanisms, allowing hydrothermal vent animals to maintain their Cu concentrations relatively constant in muscle tissues, while it accumulates in other tissues, like the gill or digestive gland.

The high Zn concentrations in the *R. exoculata* juveniles compared to the adults is unusual. In general, mesopelagic decapod crustaceans, adults and juveniles present similar Zn concentrations (Rainbow and Abdennour, 1989). Moreover, the larval development of *R. exoculata* being pelagic (Pond et al., 1997), juveniles have spent a shorter time in the presence of high Zn fluid concentrations compared to adults. Higher Zn concentrations in juveniles might be almost entirely due to adsorbed zinc, given the

higher surface area to volume ratios of juveniles compared to adults, and the proportionally large percentage of the surface that is permeable and allows Zn uptake into the body. The assimilation efficiencies (AE) are enhanced in juveniles compared to adults. This has been previously shown for Zn in cuttlefish (AE juv = 63% and adults = 41%) (Bustamante et al., 2002). It takes some time for regulatory mechanisms to equilibrate the metal concentrations and to increase Zn excretion to balance Zn uptake. Trace metals can also be accumulated from other sources, such as food, by the absorption of bioavailable forms of the metal after digestion in the alimentary tract (Rainbow et al., 1990; Wallace and Lopez, 1997). In fact, minerals are found in the animal digestive tract (Tables 4 and 5). Therefore, Cu and Zn rich minerals that are ingested and pass through the entire digestive process could also provide trace elements to the animals (Reinfelder et al., 1998). More stomach analyses from the different sites would need to be done in order to observe a pattern and to assess the bioavailability of those metals in these particles. At present it is impossible to understand the influence of the environmental particles and the trophic regime of the different samples. The availability of these minerals in the stomach, as well as the presence of metals in the water column, account for the high concentrations found in some tissues of vent organisms.

The high Hg concentrations found in the crustacean stomachs are mostly probably due to sulphides, such as cinnabar (HgS), which is an epithermal mineral formed at low temperatures. Metals in transit through the gut can result in high concentrations in the gut tissues, but do not necessarily transfer efficiently to the rest of the body (Craig et al., 1998). There is a need for research on effective metal transfer from one food link to another in order to understand the degree of efficiency of food transfer of trace metals.

No Hg mineral was found, but this might be due to the low sensitivity of the EDAX technique in relation to this metal. The Hg concentrations are low in the muscle of almost all species studied. This metal does not seem to be bioamplified along the trophic levels studied in this work, but there is a tendency for a slight increase along the crustacean food link. The mussel link is rather complex due to bacterial endosymbiosis and due to the unclear role of the commensal worm. This worm seems to feed on the mussel, but metal analyses suggests that the tissue types preyed on are not the gills or the digestive gland, since these tissues contain far higher Hg concentrations than that found in the commensal worm.

The high concentrations of Hg found in the digestive gland of the mussels, crabs and some of the shrimps might be a strategy of accumulation with temporary detoxified storage and excretion. According to Depledge and Rainbow (1990) certain tissues, like the midgut gland, digestive gland, etc., might play this role. The gill of the mussel and the mouthpart of the shrimp *R. exoculata* have higher concentrations of Hg than other tissues. The gill also presents high concentrations of Cu and Zn. Gills represent a natural pathway for metals dissolved in seawater (Bustamante et al., 2002). Moreover, these two tissues are linked with bacteria. The good metal binding properties of bacterial exopolysaccharides from deep-sea hydrothermal vents (Loaec et al., 1998), might explain the high concentrations in the shrimp mouth part (tissue covered with many bacteria) compared to the concentrations in other tissues. With regard to the endosymbiosis of the mussel gill, bacteria might have a special role, since according to Ford et al. (1995), once inside a bacteria cell, a metal can undergo enzymatic transformation, which may render it less toxic. An example is the reduction of Hg^{2+} to Hg^0 by the mercuric reductase, an enzyme that appears to be widely distributed in the bacterial kingdom. Further investigation is needed to understand these processes, in particular, with regard to the speciation of Hg in these tissues.

4.2. Bioamplification in hydrothermal food chains

Taking into account that Hg is bioamplified in marine food webs, similar tissues of animals, (with the exception of the commensal worm), of different trophic levels were chosen in order to study its transfer along the food chain. Using the same type of tissue helps to reduce the variability in contaminant data within organisms (Gray, 2002). Unfortunately, cruising conditions did not permit extended sampling in the Menez Gwen hydrothermal vent field. Therefore, biomagnification cannot be inferred from the present results.

Distinct concentrations can be observed in both food links, the mussel path and the shrimp path, with the shrimps presenting the lowest Hg values. There is an increase in Hg concentrations from mussel foot to commensal worm, and from shrimp species to the crab. However, the differences are not statistically significant. When comparing Lucky Strike and Rainbow, Hg concentrations for the same groups are within the same range. However, Hg concentrations in the shrimps from Rainbow were different from those found at Lucky Strike. The first trophic link does not present significant

differences when comparing metal concentrations between Lucky Strike and Rainbow specimens, but the differences are significant ($p < 0.05$) when specimens in the second trophic link (crustaceans) are compared.

Cu and Zn concentrations in the different species presented the same pattern of increase from prey to consumer. Cu and Zn concentrations increase from mussel mantle to commensal, and are clearly distinct from shrimps to crab, with the exception those collected from the Rainbow vent field, where the Zn concentrations in juveniles are very high compared to concentrations in the adults and the crab.

4.3. Trophic transfer

The general complexity of most food chains requires a realistic approach to biomagnification studies. This approach consists in looking at just two levels at a time (Wang, 2002). Patterns of metal transfer can only be revealed if the different trophic levels are known, at least for the metals Hg and Zn (Wang, 2002). Knowledge of the trophic levels allows us to hypothesize that there is biomagnifications of Hg, Zn and possibly Cu in the crustacean link, while on the mussel path, biomagnification is not evident. To understand the biomagnification, there is a need to study the food chain transfer factor, which is dependent on ingestion rate and assimilation efficiency. At the present time, these parameters are not known for the vent species studied.

Biomagnification of mercury in food chains is due to organic mercury. The lack of biomagnification may have been due to two factors. It may be that biomagnifications in invertebrates is different from that of vertebrates (most studies have been done on birds, fish and mammals). The other possibility is that a low methylation rate is present in the studied vent field environments. Despite several bacteria are able to methylate Hg, at ambient sulphide levels exceeding a critical level, sulphide inhibits Hg methylation (Jackson, 1998). Further investigation on the food chains with a special attention to organic and inorganic Hg should be carried out.

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Table 1. Endmember concentrations in the Menez Gwen, Lucky Strike and Rainbow vent fluids, adapted from Douville et al. (2002), Charlou et al. (2000), Charlou et al. (2002).

Site	Menez Gwen	Lucky Strike	Rainbow
Depth (m)	840	1700	2280
T (°C)	265–284	152–333	360–365
pH	4.2–4.8	3.5–4.9	2.8–3.1
H ₂ S (mM)	1.3–1.82	0.6–3.3	1–2.5
Fe (mg l ⁻¹)	1.3–1.6	1.7–48	1339
Mn (mg l ⁻¹)	3.2–3.7	4.2–24.7	123
Cu (µg l ⁻¹)	40–180	60–1650	8900
Zn (mg l ⁻¹)	0.16–0.33	0.33–3.79	10.5
Cd (µg l ⁻¹)	1.01–1.34	2.02–8.85	14.6
Pb (µg l ⁻¹)	4.4–11.6	7.2–26.9	30.6

Table 2. Location, size, depth and trophic position of the studied species.

Species	Depth (m)	Location	Trophic position	Major diet	
Annelid	<i>Brachipolynoe seepensis</i>	1700 2300	Lucky Strike Rainbow	Secondary consumer	Mussel products
Bivalve mollusc	<i>Bathymodiolus azoricus</i>	840 1700 2300	Menez Gwen Lucky Strike Rainbow	Primary consumer	Symbionte
Decapod crustacean	<i>Mirocaris fortunata</i>	840 1700 2300	Menez Gwen Lucky Strike Rainbow	Consumer	^a Mixotroph ^a ^a
Decapod crustacean	<i>Chorocaris chacei</i>	1700	Lucky Strike	Consumer	Crustacean ^a
Decapod crustacean	<i>Rimicaris exoculata</i> (juv)	2300	Rainbow	Primary consumer	Detritus?
Decapod crustacean	<i>Rimicaris exoculata</i> (adult)	2300	Rainbow	Primary consumer	Bacteria ^a
Decapod crustacean	<i>Segonzacia mesatlantica</i>	840 1700 2300	Menez Gwen Lucky Strike Rainbow	Predator	Crustacean ^a
Decapod crustacean	<i>Chaceon affinis</i>	850	Menez Gwen	Predator	Mussel and fish ^a

The major diets are based on stomach content observations. Some of them are not published yet.

^a Colaço, 2001. Trophic Ecology of deep-sea hydrothermal vent fields from the Mid Atlantic Ridge.

Table 3. Hg, Cu, and Zn concentrations for the different studied specimens.

Species	Location	Tissue	n	Hg	n	Cu	n	Zn
<i>Brachipolynoe seepensis</i> (commensal worm)	Lucky Strike	Whole	16	0.4 ± 0.2	3	29 ± 9	3	219 ± 43
	Rainbow	Whole	9	0.2 ± 0.2	4	45 ± 46	4	304 ± 325
<i>Bathymodiolus azoricus</i> (mussel)	Menez Gwen	dg	20	4.6 ± 1.3	1	22	1	40
		Gills	17	4.4 ± 1.0	9	47 ± 7	9	111 ± 42
		Mantle	20	0.8 ± 0.6	9	10 ± 7	9	61 ± 24
		Foot	25	0.8 ± 0.7	0		0	
	Lucky Strike	dg	9	2.4 ± 0.8	1	52.02	1	201
		Gills	7	4.1 ± 0.8	6	70 ± 16	6	555 ± 254
		Mantle	13	0.4 ± 0.4	4	13 ± 4	4	68 ± 14
		Foot	10	0.2 ± 0.2	0		0	
	Rainbow	dg	8	1.3 ± 0.9	7	18 ± 7	7	80 ± 51
		Gills	11	1.0 ± 0.4	8	67 ± 21	8	106 ± 38
		Mantle	9	0.3 ± 0.2	9	4 ± 2	9	46 ± 22
		Foot	12	0.3 ± 0.3	0		0	
<i>Mirocaris fortunata</i> (shrimp)	Menez Gwen	dg	1	1.3	0		0	
		Muscle	2	0.5 ± 0.05	0		0	
	Lucky Strike	dg	1	0.7	0		0	
		Muscle	1	0.08	1	46	1	58
	Rainbow	Muscle	2	1.0 ± 1.6	0		0	
<i>Chorocaris chacei</i> (shrimp)	Lucky Strike	dg	7	1.4 ± 2.0	3	264 ± 33	3	233 ± 115
		Muscle	7	0.06 ± 0.05	4	44 ± 10	4	50 ± 4
<i>Rimicaris exoculata</i> (juv) (shrimp)	Rainbow	dg	6	0.11 ± 0.069	5	107 ± 50	5	1004 ± 753
		Muscle	7	0.01 ± 0.01	6	62 ± 10	6	228 ± 96
<i>Rimicaris exoculata</i> (adult) (shrimp)	Rainbow	dg	3	0.14 ± 0.11	2	109 ± 6	2	207 ± 200
		Muscle	8	0.17 ± 0.38	5	52 ± 7	5	58 ± 10
<i>Segonzacia mesatlantica</i> (hydrothermal crab)	Menez Gwen	dg	2	1.40 ± 0.43	1	150	1	439
		Muscle	3	0.67 ± 0.33	3	47 ± 7	3	111 ± 42
	Lucky Strike	dg	6	0.35 ± 0.23	4	2840 ± 1238	4	133 ± 68
		Muscle	7	0.34 ± 0.40	4	125 ± 67	4	268 ± 37
	Rainbow	dg	8	0.20 ± 0.16	4	2850 ± 3329	4	108 ± 36
		Muscle	6	0.22 ± 0.15	4	125 ± 27	4	271 ± 15
<i>Chaceon affinis</i> (deep-sea crab)	Menez Gwen	dg	1	0.8	1	454	1	167
		Muscle	2	0.77 ± 0.28	1	33	1	221

Number of specimens (n) and mean with standard deviation metal concentration ($\mu\text{g.g}^{-1}$ dry weight) in the hydrothermal vent organisms studied for each hydrothermal vent field. (dg) stands for digestive gland.

Table 4. Minerals observed inside the stomachs of: the polychaete *Amathys lutzi*; the shrimps *Mirocaris fortunata* and *Rimicaris exoculata*; the mussel *Bathymodiolus azoricus*.

Minerals	Composition	Temperature of formation	Rainbow	Lucky Strike	Menez Gwen
Anhydrite	CaSO ₄	High	<i>Rimicaris exoculata</i>	<i>Mirocaris fortunata</i>	<i>Bathymodiolus azoricus</i>
Apatite	Ca ₅ (PO ₄) ₃ (OH,F,Cl)	Low	<i>Amathys lutzi</i>		
Baryte	BaSO ₄	Low	<i>Bathymodiolus azoricus</i>		<i>Bathymodiolus azoricus</i>
Chalcopyrite	CuFeS ₂	High	<i>Rimicaris exoculata</i>		<i>Bathymodiolus azoricus</i>
Goethite	αFeOOH	Low	<i>Amathys lutzi</i>		
Isocubanite	Altered pyrite	High	<i>Rimicaris exoculata</i>		
Pyrite	FeS ₂	High	<i>Rimicaris exoculata</i>	<i>Bathymodiolus azoricus</i>	<i>Mirocaris fortunata</i>
Pyrite cubic	FeS ₂	High	<i>Bathymodiolus azoricus</i>		
Pyrite framboidal	FeS ₂	Low			<i>Bathymodiolus azoricus</i>
Pyrite low temperature	FeS ₂	Low	<i>Rimicaris exoculata</i>	<i>Mirocaris fortunata</i>	
Pyrrhotite	Fe ₇ S ₈	High	<i>Rimicaris exoculata</i>	<i>Amathys lutzi</i>	
Silica	Si		<i>Bathymodiolus azoricus</i>	<i>Mirocaris fortunata</i>	
Silica amorphous		Hydrothermal deposits	<i>Rimicaris exoculata</i>		
Sphalerite	ZnS	Low	<i>Rimicaris exoculata</i>	<i>Amathys lutzi</i>	<i>Mirocaris fortunata</i>
Sulphur	S	Secondary product	<i>Bathymodiolus azoricus</i>	<i>Mirocaris fortunata</i>	

Table 5. Hg, concentrations for the stomach of the studied specimens.

Species	Location	Tissue	<i>n</i>	Hg
Shrimp <i>Mirocaris fortunata</i>	Lucky Strike	Stomach	1	5.03
Shrimp <i>Chorocaris chacei</i>	Lucky Strike	Stomach	6	3.2 ± 3.6
Shrimp <i>Rimicaris exoculata</i> (juv)	Rainbow	Stomach	5	1.69 ± 1.38
Shrimp <i>Rimicaris exoculata</i> (adult)	Rainbow	Mouthparts	9	0.38 ± 0.28
Crab <i>Segonzacia mesatlantica</i>	Lucky Strike	Stomach	2	4.25 ± 5.19
	Rainbow	Stomach	2	1.11 ± 1.20

Number of specimens (*n*) and mean with standard deviation metal concentration ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) in the hydrothermal vent organisms studied for each hydrothermal vent field.