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Metal concentrations in the shell of *Bathymodiolus azoricus* from contrasting
hydrothermal vent fields on the Mid-Atlantic Ridge

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16

17 **ABSTRACT**

18 Specimens of *Bathymodiolus azoricus* were sampled along the Mid-Atlantic Ridge at
19 the Menez Gwen, Lucky Strike and Rainbow hydrothermal fields. Individual shells
20 (n=51), through the weight range 0.62 to 15.70g, were analysed for their magnesium,
21 strontium, iron, manganese, copper, zinc and cadmium concentrations. Amongst the
22 marine molluscs the shell of *B. azoricus* is confirmed as being particularly
23 impoverished in strontium (mean 943 $\mu\text{g g}^{-1}$). Trace metal concentrations in the shells
24 decreased in the order Fe>Mn>Zn>Cu>Cd. Despite originating from trace metal rich
25 environments mean concentrations were low (37.9, 13.2, 10.7, 1.1 and 0.7 $\mu\text{g g}^{-1}$,
26 respectively). Irrespective of geographical origin magnesium, strontium and copper
27 concentrations were primarily dictated by shell weight. In contrast cadmium
28 concentrations were elevated in shells from the Rainbow field and ambient seawater
29 chemistry imparted site-specific chemical fingerprints to the shells with respect to the
30 iron to manganese ratio.

31

32 **Keywords:** Metals, shells, *Bathymodiolus azoricus*, hydrothermal vent field, Menez
33 Gwen, Lucky Strike, Rainbow

1. Introduction

To date seven extensive hydrothermal fields have been discovered along the Mid-Atlantic Ridge (MAR) between 14°N and 38°N. Since 1991 the area has been extensively surveyed with respect to both the chemical characteristics of the hydrothermal fluids and the associated biological fauna. Areas contiguous to the hydrothermal vents are highly productive (Rousse et al. 1997, Little & Vrijenhoek, 2003, van Dover & Lutz, 2004). Among the life forms bivalve molluscs are generally present in large numbers, particularly the mussel *Bathymodiolus azoricus* (van Dover et al., 1996, von Cosel et al., 1999, Colaço et al., 1998, 2002, Desbruyères et al., 2000, 2001).

Much attention has been given to the extent to which the high metal concentrations in hydrothermal areas accumulate in the soft tissues of the associated bivalves (Roesijadi & Crecelius, 1984, Roesijadi et al., 1985, Smith & Flegal, 1989, Geret et al., 1998, Rousse et al., 1998, Company et al., 2004, 2006, Hardivillier et al., 2004, Bebianno et al., 2005, Colaço et al., 2006, Kádár et al., 2006a, b, 2007). In contrast there are few data on the extent to which the high environmental trace metal concentrations influence the chemical assemblage in the shell (Roesijadi & Crecelius, 1984; Roesijadi et al., 1985, Kádár & Costa, 2006, Cravo et al., 2007).

Mineralisation of skeletal carbonates involves the deposition of calcium carbonate in the form of calcite and/or aragonite. The minor elements, magnesium and strontium, together with the trace metals are incorporated into the carbonate crystal lattice of the shell by diadochically replacing the calcium ions in the calcite and aragonite (Deer et al., 1966).

Roesijadi & Crecelius (1984) and Roesijadi et al. (1985) were the first authors to report on the chemical composition of shells taken from hydrothermal areas. They

presented data on the trace metal concentrations in the shell of five specimens of the vent clam *Calymene magnifica* taken from the Oasis site (21°N, 109°W) on East Pacific Rise. The very low mean concentrations of Fe, Mn, Cu, Zn and Cd in these shells (17.4, <3, 5.9, 3.4 and 6.9 $\mu\text{g g}^{-1}$ respectively) lead the authors to conclude that, in contrast to the soft tissues of *Calymene magnifica*, its shell was not a site of metal accumulation or deposition. Kádár & Costa (2006) and Cravo et al. (2007) provided preliminary observations on the composition of the shell of *B. azoricus* taken from the MAR. Both these works provide valuable but limited information on the composition of the shell and its variability. In an inter-population study Kádár & Costa (2006) generated data on five individual shells taken from each of two contrasting hydrothermal sites along the ridge. Cravo et al. (2007) provided data on a greater number of individual shells but these were all from a single site. Interestingly the two works produced a dichotomy of opinion with respect to two key issues. Kádár & Costa (2006) reported high concentrations of trace metals in the shell (mean concentrations of iron, copper and zinc were 280, 80 and 40 $\mu\text{g g}^{-1}$ respectively). In contrast Cravo et al. (2007) found that despite originating from a trace metal rich environment the trace metal concentrations in the shell of *B. azoricus* were exceptionally low, the mean concentrations of iron, copper and zinc being 20.6, 0.6 and 9.4 $\mu\text{g g}^{-1}$ respectively. The disparity with respect to the levels of trace metals can in part be explained by the different cleaning and analytical procedures adopted. Secondly, Kádár & Costa (2006) concluded that the shell of *B. azoricus* was a good monitor of environmental levels of iron, copper and zinc in waters contiguous to deep-sea hydrothermal vents. In contrast, Cravo et al. (2007) inferred that due to the low metal concentrations found the shell of *B. azoricus* had little potential use for trace metal monitoring in hydrothermal vent areas.

The present paper extends the observations of Kádár & Costa (2006) and Cravo et al. (2007) and provides the first comprehensive data on both the intra- and inter-

population variability in the chemical composition of the crystal matrix of the shell of *B. azoricus* taken from contrasting hydrothermal fields along the MAR.

2. Study Area

Samples of *B. azoricus* were taken from vent fields on the MAR in the vicinity of the Azores Triple Junction (Fig. 1). There are three major hydrothermal fields in the area, Menez Gwen, Lucky Strike and Rainbow. Menez Gwen (37°51' N, 31°31' W) is the northernmost and the shallowest of the three with a depth of 850 m (Company et al., 2004). To the south of Menez Gwen is Lucky Strike (37°17'-37°18'N, 32°16'-32°17'W). This field, discovered in 1992, is one of the largest known active fields on the MAR. It is deeper than Menez Gwen. Depths vary from 1630 to 1730 m (mean 1700 m, Charlou et al., 2000). The hydrothermal fields are distributed around 37°N and the discrete active venting sites are dispersed over an area about 700 m long and 300 m wide. To the southeast and the northwest the hydrothermal venting sites are distributed around a lava lake. Within this large hydrothermal area samples of *B. azoricus* were taken from two locations. In the active zone to the northwest, samples were taken adjacent to Bairro Alto (37°17'34"N, 32°17'W). To the southeast of the lava lake further samples of *B. azoricus* were collected in proximity to Eiffel Tower (37°17'19"N, 32°16'29"W). Further south along the ridge from Lucky Strike is Rainbow (Fig. 1). Located at 36°13'N, 33°54'W and discovered in 1997 (Fouquet et al., 1997) this field is the deepest active segment of the Azores Triple Junction with water depths between 2270-2320 m (mean 2300 m, Charlou et al., 2002).

Data on the physical and chemical properties of the hydrothermal fluids in the area of the MAR have been provided by Fouquet et al. (1995, 1997), Charlou et al. (1997, 2000, 2002), Douville et al. (1997, 1999, 2002), Langmuir et al. (1997), von Damm et al. (1998), Sarradin et al. (1999), Desbruyères et al. (2001). Due to the geographical

remoteness and difficulties of sampling, these data provide only snapshot descriptions, fixed in both space and time, and provide no detailed information on the temporal variability in chemical composition of the fluids or detailed information upon the spatial variability in the composition of different fluids within the same hydrothermal field. It is nevertheless possible to draw a number of general conclusions and inferences from those data currently available.

Comparative data on the chemistry of the vent fluids at Menez Gwen, Lucky Strike and Rainbow are given in Table 1. These data have been adapted from those provided by Douville et al. (2002) and are extrapolated to $Mg = 0 \text{ mmol kg}^{-1}$ (the standard method of reporting vent fluid composition to correct for variable amounts of sea water entrainment during sampling). The most striking feature of the data is the marked differences in both physical and chemical properties of the fluids at the three sites and particularly at Rainbow. In contrast to Menez Gwen and Lucky Strike, the Rainbow field is ultramafic. The vent fluids are very acidic ($pH < 3$) and have very high temperatures ($> 350^\circ\text{C}$). The predominant gaseous emission is methane rather than hydrogen sulphide and the vent fluids are very enriched in metals compared to Lucky Strike and Menez Gwen. This is particularly pronounced with respect to the concentrations of iron and manganese but is also clearly evident with respect to the concentrations of copper, zinc and cadmium. In contrast the physical and chemical characteristics of the vent fluids at Menez Gwen and Lucky Strike exhibit a reasonable degree of similarity. There is however the tendency for all the trace metals, iron, manganese, copper, zinc and cadmium to be in greater concentrations in the vent fluids at Lucky Strike.

From the data currently available it is reasonable to infer that trace metal enrichment in the areas contiguous to the hydrothermal vents along the MAR will be in the order Rainbow > Lucky Strike > Menez Gwen.

3. Materials and Methods

3.1. Collection

Specimens of *B. azoricus* were collected from the Menez Gwen, the Lucky Strike field at Bairro Alto and Eiffel Tower and also from the Rainbow field. Collection was by a remotely operated vehicle VICTOR during ATOS cruise in June to July 2001 within the scope of the European Scientific Research Project – Ventox (Sarradin et al., 2001). From the limited amount of material available from each site specimens of *B. azoricus* were selected through the available size range of each population. Damaged shells were rejected.

3.2. Sample Cleaning

The typical state of the surface of the specimens of *B. azoricus* before cleaning is illustrated in Fig. 2.1. A detailed exposition of the problems of cleaning shells for subsequent trace metal analysis has been provided by Foster & Chacko (1995). Two issues are of particular relevance to the cleaning of the *B. azoricus* specimens from the MAR. First, the shells have a periostracum which may be present in various states of completion from shell to shell. The periostracum is extremely enriched in trace metals compared to the underlying crystal matrix (Kádár & Costa, 2006). Secondly, the vent areas are very rich in trace metals in both the particulate and colloidal states and these materials both adhere and adsorb onto the surface of the shells, whether this be the periostracum or the outer crystal matrix of the shell where the peristracum is missing or ruptured. If minute traces of either the periostracum or the adhered and/or adsorbed particulate and colloidal materials remain after cleaning they will grossly distort the analytical data. Mechanical cleaning alone cannot guarantee a perfectly clean shell surface. This can only be done by submitting each shell individually to a selective chemical attack designed to remove all material from the shell surface which is not an integral component of the crystalline shell matrix.

At the laboratory the soft parts were removed and the shells washed in a jet of distilled water. They were then scrubbed in distilled water with a toothbrush to remove loosely attached biogenic and inorganic particles. Subsequent to this preliminary cleaning the shells were dried at 80 °C to constant weight. Each individual shell pair was then subjected to selective chemical attack as detailed by Foster & Chacko (1995).

Each pair of shells was individually treated with 5% w/v hydrogen peroxide in the ratio 10 mL g⁻¹ shell weight and gently heated at 60 °C for 48 hours. After rinsing with deionised distilled water, 0.005M HCl was added, again in the ratio of 10 mL g⁻¹ shell weight and the samples left for 48 hours. For both these stages of treatment shells were placed in vials of appropriate diameter to ensure that the reagents covered and therefore reacted with the entire surface of the shell. The shells were then washed in distilled deionised water and oven dried. The effect of the selective chemical attack is illustrated in Fig. 2. Subsequent to the treatment the shells of *B. azoricus* were a uniform brilliant white. Each shell pair was then pulverised in an agate mortar and the resulting homogenous fine powders stored for subsequent analysis.

3.3. Analytical procedure

Duplicate samples of each shell (0.1 g) were totally digested in a mixture of concentrated nitric acid and 50% w/v hydrogen peroxide. After evaporation to dryness, the residue was redissolved in 2 mL of concentrated hydrochloric acid and fumed to dryness. The final residue was redissolved in 10 mL of 0.05M hydrochloric acid. Sub samples of this digest were taken for the analysis of Mg, Sr, Fe, Mn, Zn and Cu by flame atomic absorption spectrophotometry (Perkin-Elmer, model Analyst 800). All analyses were calibrated against analytical standards containing the stoichiometric equivalent of spectrographically pure calcium carbonate (HCl digested) as the samples being analyzed and the samples were batch-analyzed in random

sequence with respect to both the original weight of the shell specimens and geographical origin in order to remove any analytical bias from the intra-population and inter-population studies.

In the absence of an analytical reference material for marine biogenic carbonate all analyses performed were calibrated against an internal reference standard consisting of a homogeneous fine powder of the shells of five cleaned specimens of *B. azoricus*. The mean concentrations in $\mu\text{g g}^{-1}$ ($\pm$ standard error) from 10 individual analyses of this powder were: Mg: 473 ± 2.5 , Sr: 868 ± 7.3 , Fe: 20.9 ± 1.0 , Mn: 4.0 ± 0.2 , Zn: 9.3 ± 0.2 , Cu: 0.7 ± 0.1 , Cd: 0.6 ± 0.2 .

3.4. Statistical analysis

Principal Component Analysis (PCA) was applied to the data matrix of shell weight and the measured concentrations of the minor and trace elements. The coefficient of correlation (r) between these variables was calculated at a level of significance of 99%. In order to assess the differences in metal concentrations between populations, the one-way ANOVA was applied for metal concentrations fitting normal distributions. Whenever metal concentrations did not fit to normal distributions Kruskal-Wallis test was used. Both tests used a minimum level of significance of 99%.

4. Results and Discussion

4.1. Intra-population variability

Summary data on the minor and trace metal concentrations in the shells of *B. azoricus* collected at each of the four sampling sites along the MAR are given in Table 2. Reference to the range of concentrations and the % coefficients of variation shows that for all the elements there was considerable variability from shell to shell within all four populations. Variability in concentration from shell to shell within each

population was generally lower with respect to the minor elements, magnesium and strontium. Of the trace elements variability in concentration of iron from shell to shell within each population was generally less than that exhibited by manganese, zinc, copper and cadmium.

Studies revealing the heterogeneity in chemical composition that can develop amongst the shells within the population of an individual species from a single location have been conducted by Babukutty & Chacko (1992), Foster & Chacko (1995), Foster et al. (1997), Cravo et al. (2002, 2007). The unifying characteristic of these studies is the very marked differences in chemical composition from shell to shell that can develop within a single population. Marked variability in composition from shell to shell is present within populations taken from pristine environments (Foster & Chacko, 1995, Cravo et al., 2002) and can be significantly amplified in populations taken from environments which are less pristine. This consideration is of particular relevance to the data sets presented in Table 2. The shells contributing to each population were taken from locations subjected to extreme environmental variability with respect to both steep concentration and physico-chemical gradients.

A detailed analysis of the intra-population variability in chemical composition of the shell of *B. azoricus* from Menez Gwen (n=21) was presented by Cravo et al. (2007). A linear least square correlation matrix amongst the variables of shell weight and the measured concentrations of the minor and trace elements produced only one correlation of significance, namely that between strontium and magnesium ($r = +0.55$). Corresponding correlation matrices for the much smaller shell populations (n= 10) taken from Eiffel Tower, Bairro Alto and Rainbow produced no correlations of statistical significance. The heterogeneity in shell composition within these populations is illustrated in Table 3 and Fig. 3 with respect to shell weight and the

concentrations of magnesium, strontium, iron, manganese, zinc, copper and cadmium in the shells taken from the Rainbow vent field.

4.2. Inter-population variability

Within each population there was considerable variability in the minor and trace metal concentrations from shell to shell. Despite the intra-population variability in the chemical composition of the shells at each of the sampling locations there were statistically significant inter-population differences ($p < 0.01$) in the composition with respect to the concentrations of several of the trace metals. The mean concentrations (+ standard deviations) of iron, manganese, zinc and cadmium associated with the shells from Menez Gwen, Eiffel Tower, Bairro Alto and Rainbow are compared in Fig. 4.

Data on the chemistry of the hydrothermal fluids at the various sites (Table 1) identified the hydrothermal fluids issuing from the vents at Rainbow to be particularly rich in iron, manganese, copper, zinc and cadmium compared to those at Lucky Strike and Menez Gwen. The magnitudes of the iron and manganese concentrations in the shells from Rainbow were not exceptional compared with those in the shells from other sites. These two elements did however exhibit particularly pronounced environmentally induced modifications in shell concentrations (Figs. 4a and 4b). At all four sampling sites there were statistically significant differences in iron concentrations associated with the shells ($p < 0.01$). Concentrations increased in the order Menez Gwen < Bairro Alto < Rainbow < Eiffel Tower from the low mean concentrations associated with the shells from Menez Gwen ($20.6 \mu\text{g g}^{-1}$) to a mean of $55.0 \mu\text{g g}^{-1}$ in the shells at Eiffel Tower. As with iron, all of the four shell populations were significantly different ($p < 0.01$) with respect to their manganese concentrations (Fig. 4b). However, for manganese concentrations increased in the order Menez Gwen < Eiffel Tower < Rainbow < Bairro Alto. Minimum mean

concentrations were again associated with the shells from Menez Gwen ($3.7 \mu\text{g g}^{-1}$) but in the case of manganese the shells taken from Bairro Alto had the highest mean concentration ($31.4 \mu\text{g g}^{-1}$), almost one order of magnitude greater than that found at the former place.

The contemporaneous changes in shell chemistry with respect to iron and manganese produced distinctive chemical signatures uniquely identifying the geographical origin of the shells. The signatures were particularly distinctive with respect to the shells taken from the sampling sites at Lucky Strike. Reference to Table 2 shows that the chemical composition of the crystal matrices of the shells taken from Eiffel Tower and Bairro Alto were extraordinarily congruent. There were no statistically significant differences ($p > 0.01$) between the shell populations with respect to their magnesium, strontium, zinc, copper and cadmium concentrations. In contrast shells from Eiffel Tower were richer in iron and very much impoverished in manganese compared with the shells taken from Bairro Alto. As a consequence shells from Eiffel Tower had a Fe/Mn ratio > 5 and were uniquely identified from the shells taken from Bairro Alto which had a Fe/Mn ratio < 2 .

Interestingly, at Lucky Strike the presence of two distinct source waters have been reported (von Damm et al., 1998, Charlou et al., 2000, Desbruyères et al., 2001). The temperature and chemical compositions of the fluids issuing from the vents to the northwest (Bairro Alto) are significantly different from those to the south (Eiffel Tower). Whatever differences do exist they are not reflected in the chemical assemblage of the crystal matrix of *B. azoricus* taken from the two sites with respect to the magnesium, strontium, copper, zinc and cadmium concentrations. This is in sharp contrast to the unique fingerprints associated with the two shell populations with respect to the Fe/Mn ratio. It is however unlikely that the differences in the iron and manganese concentrations in the shells taken from Bairro Alto and Eiffel Tower,

or indeed the differences in iron and manganese content of the shells from any of the sites (Figs. 4a and b) are a direct reflection of differences in the iron and manganese content of the venting source waters. The solution chemistry of iron and manganese in the waters contiguous to the hydrothermal fluids will be primarily dictated by the considerable hydrogen ion and electron activity gradients and the kinetics of both the thermodynamically spontaneous oxidative redox reactions and subsequent changes of state which the gradients induce.

In contrast to iron and manganese there was very little difference in the mean zinc concentrations amongst the shells from all four sites (Fig. 4c). The only element to show preferential enrichment in the shells taken from Rainbow was cadmium (Fig. 4d).

4.3. General characteristics of shell chemistry of *B. azoricus* from the MAR

In all a total of 51 specimens of *B. azoricus* were taken from the four contrasting sites along the MAR and analyzed individually. Summary data relating to the concentrations of the minor and trace metals in these 51 specimens are given in Table 4. The most striking feature of the data in Table 4 is that the degree to which both the minor and trace elements replace the Ca^{2+} ions in the crystal matrix of the shell of *B. azoricus* is very low compared to that which can occur in shells of coastal water analogues. The minor element concentrations (mean Mg and Sr concentration: 407 and 943 $\mu\text{g g}^{-1}$ respectively) are exceptionally low.

Magnesium concentrations in the shell of marine molluscs can vary over the range 90 $\mu\text{g g}^{-1}$ (Foster & Cravo 2003) to 6144 $\mu\text{g g}^{-1}$ (Afaj & Elewe 1989). In general aragonitic shells have low magnesium concentrations within the range 90 to 500 $\mu\text{g g}^{-1}$. Magnesium concentrations are consistently lower than those of strontium, the aragonitic shells being characterised by an Mg/Sr ratio of <0.5 (Foster & Cravo

2003). Calcitic shells exhibit much higher concentrations over a wider range and concentrations above $3500 \mu\text{g g}^{-1}$ are not unusual. The magnesium concentrations are generally much higher than those of strontium and the Mg/Sr ratio is generally >1 . In species with bi-mineral shells the magnesium concentrations are of intermediate values depending on the relative amounts of calcite and aragonite contributing to the shell crystal matrix. The shells of *Mytilus* species are of mixed mineralogy (Wilbur 1972, Milliman 1974, Lowenstam and Weiner 1989). Consistent with the *Mytilidae* the shell of *B. azoricus* is also of mixed mineralogy (Kádár & Costa 2006). To date however there are no quantitative data on the relative contributions of the calcite and aragonite polymorphs. The magnesium concentrations are very low for a shell of mixed mineralogy and is easily within the range of concentrations exhibited by purely aragonitic shells.

Mineralogy plays only a secondary role in controlling strontium concentrations associated with the shell of marine molluscs (Harriss, 1965) and concentrations generally fall in the range 950 to $3500 \mu\text{g g}^{-1}$. Isolated examples of concentrations $<950 \mu\text{g g}^{-1}$ do exist (Afaj & Elewe 1989, Foster & Cravo 2003). The low strontium concentrations with values falling to as low as $536 \mu\text{g g}^{-1}$, confirms this species as having one of the most strontium impoverished shells amongst the marine molluscs.

Both magnesium and strontium are major elements in seawater. The above data indicates that with respect to the shells of marine molluscs in general both these elements find it particularly difficult to substitute for Ca^{2+} ions in the crystal matrix of the shell of *B. azoricus*. Reference to Table 4 indicates that this difficulty is not unique to the minor elements. Although the shells originate from hydrothermal trace metal rich environments the trace metal concentrations in the shells are also low. The mean trace metal concentrations in the crystal matrix of *B. azoricus* decrease in the order $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Cd}$ from a maximum mean concentration of $37.9 \mu\text{g g}^{-1}$ with

respect to iron down to $0.7 \mu\text{g g}^{-1}$ in the case of cadmium. The magnitude of these concentrations compare unfavourably with those reported for the shells of coastal marine molluscs. In *Mytilus edulis* for example iron and manganese concentrations as high as 5100 and $130 \mu\text{g g}^{-1}$ have been reported (Szefer & Szefer 1985), copper concentrations as high as $160 \mu\text{g g}^{-1}$ were observed by Al-Dabbas et al. (1984) and Bertine & Goldberg (1972) found shell concentrations of zinc up to $39 \mu\text{g g}^{-1}$. There are numerous examples in the literature of very much higher concentrations than those given above for the shell of species other than *Mytilus edulis*.

4.4. Non site specific trends in the shell chemistry of *B. azoricus* from the MAR

The inter-population comparison of shell composition showed that differences in ambient seawater chemistry imparted site specific chemical fingerprints to the crystal matrix with respect to the concentrations of iron, manganese and cadmium (Fig. 4). Ambient seawater chemistry is however only one of many factors that can influence the chemical composition of the shell. For a particular species parameters such as size, age, sex, stage of growth, rate of growth and diet may all variously influence the composition of shell. As a first approximation it has proved useful to equate the single parameter of shell weight as synonymous with the multitude of agencies that may variously influence the growth of the shell (Foster & Chacko, 1995; Cravo et al., 2002).

The summary data given in Table 4 for 51 specimens of *B. azoricus* were taken from various sites along the MAR. The individual chemical composition of these 51 specimens together with the individual weight of the shells provide an ideal data matrix from which to detect any general changes in shell composition that are primarily induced by factors relating to the weight of the shell rather than site specific differences in ambient seawater chemistry.

PCA was applied to the 51 columns, 8 rows (shell weight and concentrations of magnesium, strontium, iron, manganese, zinc, copper and cadmium) data matrix generated from the individual analyses of the shells of *B. azoricus* from the MAR. The principal axis accounted for 44.7% of the total variance in the data. The dominant characteristic on this axis with a loading of 0.93 was the weight of the shell. In other words, shell weight may reflect age or growth related agencies as the dominant factors inducing changes in chemical composition amongst the shells, rather than any differences in chemical composition in the waters at their sites of collection. A linear least squares correlation matrix of the same 51 columns, 8 rows data set generated a number of statistically significant relationships suggesting growth as a dominant controlling variable upon the chemical composition of the shells. The relationships between the weight of shell and the strontium concentration, the Mg/Sr ratio, copper concentration and zinc concentration are shown in Fig. 5.

The concentrations of both minor elements in the shell were influenced by shell weight. Magnesium concentrations decreased and strontium concentrations increased (Fig. 5a) with increasing shell weight. As a consequence there was also a strong correlation ($r = -0.61$) between the Mg/Sr ratio and shell weight, the ratio showing a steady progressive decrease as shell weight increased (Fig. 5b). In addition the most statistically significant relationship ($r = +0.74$) was that between shell weight and the copper concentration with the copper concentrations increasing with the weight of the shell (Fig. 5c). For comparative purposes the corresponding relationship between the shell weight and zinc concentration is shown in Fig. 5d.

The shell of *B. azoricus* is composed of both calcite and aragonite (Kádár & Costa 2006). As such it is most likely that the changes in the Mg/Sr ratio during shell growth are induced by subtle changes in the relative amounts of calcite and aragonite contributing to the crystal matrix. That the Mg/Sr ratio decreases (i.e. magnesium

concentrations decrease and strontium concentrations increase with increasing shell weight) implies that the % aragonite in the crystal matrix increases at the expense of calcite during growth.

The fact that copper concentrations in the shell progressively increase through the weight range from 0.2 to 15.70 g is unexpected, particularly in view of the contemporaneous decrease in the Mg/Sr ratio. On purely steric grounds, as the Cu^{2+} and Mg^{2+} ions both have ionic radii less than that of Ca^{2+} , decreases in the % calcite in the structure, reflected by decreases in the concentration of magnesium during growth, should if anything, be accompanied by decreases in the concentration of copper, not increases.

The heterogeneity in the chemical composition of the shells with respect to magnesium, strontium and copper were primarily induced by factors related to their respective weights. This does not imply that there are no environmental differences amongst the sites with respect to the bioavailability of these elements, or that any differences that may exist have not been reflected in the chemical composition of the shells. It does imply that, for these elements, any environmentally induced modifications in shell chemistry due to ambient seawater concentrations are much smaller and generally swamped by much larger modifications in shell chemistry induced by factors relating to the weight of the shells.

5. Conclusions

The minor and trace metal concentrations in the shell of *B. azoricus* are low. There are large variations in concentrations from shell to shell even when they originate from the same location. The heterogeneities are in part induced by shell weight and growth related factors and variations in the relative amounts of aragonite and calcite

contributing to the mineralogy of the shell. Such considerations lead Cravo et al. (2007) to infer that inter-population differences in shell composition induced by differences in ambient seawater chemistries may be swamped and lost amongst the gross heterogeneities in shell chemistry induced by other factors. The preceding data, relating specifically to the shells of *B. azoricus* within the weight range 0.62 to 15.70 g and to the chemistries of the waters contiguous to the Menez Gwen, Eiffel Tower, Bairro Alto and Rainbow vents, substantiate this inference with respect to magnesium, strontium, copper and zinc. In contrast, site-specific chemical fingerprints are imparted to the crystal matrix with respect to the concentrations of iron, manganese and cadmium. The inference is clearly not upheld with respect to these three elements.

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703 Fig. 2. Different stages of shell cleaning: 1- before washing with distilled water, 2-
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707 Figure. 3. Shell weight (g) and magnesium (a), strontium and iron (b), manganese
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711 Fig. 4. Comparison of the mean concentrations (+ standard deviations) of a) iron, b)
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716 Fig. 5. The relationship between the weight of shell and a) strontium concentration, b)
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772 Table 1. Comparative data on the physical and chemical characteristics of the
 773 hydrothermal fluids at Menez-Gwen, Lucky Strike and Rainbow (adapted from
 774 Douville et al., 2002).

Site	Menez-Gwen	Lucky Strike	Rainbow
T (°C)	271 - 284	185 - 324	365
pH	4.5	3.4 - 5.0	2.8
H₂S (mM)	1.5	0.6 - 3.4	1.0
CO₂ (mM)	17 - 20	8.9 - 28	< 16
CH₄ (mM)	1.35-2.63	0.5-0.97	2.2-2.5
Cl (mM)*	380 - 400	413 - 554	750
Si (mM)*	8.2 - 11.2	8.2 - 16	6.9
Mg (mM)*	0	0	0
Sr (μM)*	100	80-130	200
Fe (μM)*	< 2 - 18	70 - 920	24000
Mn (μM)*	59 - 68	77 - 450	2250
Cu (μM)*	< 2	< 2 - 30	140
Zn (μM)*	< 2	< 2 - 40	160
Cd (nM)*	<9 - 12	18 -79	130

775 *- Values are averaged end-members obtained by correcting the purest fluids collected from each site
 776 back to 0 mM Mg concentration
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Table 2. Summary data on the minor and trace metal concentrations in the shell of *B. azoricus* taken from four sites along the Mid-Atlantic Ridge

	Menez Gwen (n=21)				Eiffel Tower (n=10)				Lucky Strike Bairro Alto (n=10)				Rainbow (n=10)			
	mean±SD	min	max	%CV	mean±SD	min	max	%CV	mean±SD	min	max	%CV	mean±SD	min	max	%CV
Weight (g)	1.75 ± 0.57	0.62	2.63	33	6.99 ± 1.24	6.04	10.31	18	11.05 ± 2.64	6.7	15.7	24	6.24 ± 1.89	4.52	9.39	30
Mg (µg g ⁻¹)	458 ± 78	369	687	17	347 ± 68	254	471	20	378 ± 60	315	496	16	391 ± 85	288	596	22
Sr (µg g ⁻¹)	871 ± 118	536	1078	14	1028 ± 89	890	1136	9	1063 ± 182	741	1261	17	891 ± 78	727	1003	9
Mg/Sr Ratio	0.53± 0.09	0.39	0.76	16	0.34± 0.07	0.24	0.50	22	0.36± 0.08	0.25	0.47	20	0.44± 0.09	0.30	0.63	21
Fe (µg g ⁻¹)	20.6 ± 3.3	15.6	27.7	16	55.0 ± 7.8	34.0	62.1	14	44.2 ± 8.2	28.8	51.6	19	50.9 ± 12.4	30.5	61.3	24
Mn (µg g ⁻¹)	3.7 ± 3.1	0.1	10.7	84	8.6 ± 4.2	2.9	16.6	49	31.4 ± 13.0	15.3	51.1	41	19.5 ± 5.0	11.5	29.0	26
Zn (µg g ⁻¹)	9.4 ± 3.2	4.1	15.4	34	11.3 ± 4.6	6.0	19.1	41	13.0 ± 5.3	4.5	21.4	41	10.5 ± 2.0	7.3	13.3	19
Cu (µg g ⁻¹)	0.6 ± 0.4	0.1	1.4	79	1.7 ± 0.4	1.4	2.7	22	1.6 ± 0.4	1.0	2.3	24	1.2 ± 0.3	0.9	1.8	27
Cd (µg g ⁻¹)	0.6 ± 0.6	0.2	2.1	87	0.5 ± 0.3	0.2	1.0	58	0.4 ± 0.2	0.2	0.6	47	1.4 ± 0.7	0.5	2.4	48

1 Table 3. Linear least square correlation matrix between shell weight and metal
2 concentrations in the shell of *B. azoricus* taken from Rainbow (n=10).

	Shell Weight	Mg	Sr	Fe	Mn	Zn	Cu	Cd
Shell Weight	1							
Mg	-0.04	1						
Sr	0.23	0.23	1					
Fe	0.38	0.60	-0.08	1				
Mn	0.32	0.30	0.49	0.17	1			
Zn	-0.16	-0.47	-0.41	-0.16	-0.02	1		
Cu	0.52	-0.16	0.23	0.36	-0.15	-0.43	1	
Cd	-0.24	-0.31	-0.06	-0.15	0.47	0.47	-0.27	1

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5 Table 4. Shell weight (g) and minor and trace metal concentrations ($\mu\text{g g}^{-1}$) in the
 6 shell of *B. azoricus* taken from the Mid-Atlantic Ridge (n=51).

	Mean	Minimum	Maximum	% CV
Shell weight (g)	5.47	0.62	15.70	71
Mg	407	254	687	21
Sr	943	536	1261	15
Mg/Sr	0.44	0.24	0.76	26
Fe	37.9	15.6	62.1	44
Mn	13.2	0.1	51.1	95
Zn	10.7	4.1	21.4	37
Cu	1.1	0.1	2.7	57
Cd	0.7	0.2	2.4	84

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