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

3

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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

34 **1. Introduction**

35 To date seven extensive hydrothermal fields have been discovered along the Mid-
36 Atlantic Ridge (MAR) between 14°N and 38°N. Since 1991 the area has been
37 extensively surveyed with respect to both the chemical characteristics of the
38 hydrothermal fluids and the associated biological fauna. Areas contiguous to the
39 hydrothermal vents are highly productive (Rousse et al. 1997, Little & Vrijenhoek,
40 2003, van Dover & Lutz, 2004). Among the life forms bivalve molluscs are generally
41 present in large numbers, particularly the mussel *Bathymodiolus azoricus* (van Dover
42 et al., 1996, von Cosel et al., 1999, Colaço et al., 1998, 2002, Desbruyères et al.,
43 2000, 2001).

44
45 Much attention has been given to the extent to which the high metal concentrations in
46 hydrothermal areas accumulate in the soft tissues of the associated bivalves
47 (Roesijadi & Crecelius, 1984, Roesijadi et al., 1985, Smith & Flegal, 1989, Geret et
48 al., 1998, Rousse et al., 1998, Company et al., 2004, 2006, Hardivillier et al., 2004,
49 Bebianno et al., 2005, Colaço et al., 2006, Kádár et al., 2006a, b, 2007). In contrast
50 there are few data on the extent to which the high environmental trace metal
51 concentrations influence the chemical assemblage in the shell (Roesijadi &
52 Crecelius, 1984; Roesijadi et al., 1985, Kádár & Costa, 2006, Cravo et al., 2007).

53
54 Mineralisation of skeletal carbonates involves the deposition of calcium carbonate in
55 the form of calcite and/or aragonite. The minor elements, magnesium and strontium,
56 together with the trace metals are incorporated into the carbonate crystal lattice of
57 the shell by diadochically replacing the calcium ions in the calcite and aragonite
58 (Deer et al., 1966).

59
60 Roesijadi & Crecelius (1984) and Roesijadi et al. (1985) were the first authors to
61 report on the chemical composition of shells taken from hydrothermal areas. They

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

87

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

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

92

93

94 **2. Study Area**

95 Samples of *B. azoricus* were taken from vent fields on the MAR in the vicinity of the
96 Azores Triple Junction (Fig. 1). There are three major hydrothermal fields in the area,
97 Menez Gwen, Lucky Strike and Rainbow. Menez Gwen (37°51' N, 31°31' W) is the
98 northernmost and the shallowest of the three with a depth of 850 m (Company et al.,
99 2004). To the south of Menez Gwen is Lucky Strike (37°17'-37°18'N, 32°16'-32°17'W).
100 This field, discovered in 1992, is one of the largest known active fields on the MAR. It
101 is deeper than Menez Gwen. Depths vary from 1630 to 1730 m (mean 1700 m,
102 Charlou et al., 2000). The hydrothermal fields are distributed around 37°N and the
103 discrete active venting sites are dispersed over an area about 700 m long and 300 m
104 wide. To the southeast and the northwest the hydrothermal venting sites are
105 distributed around a lava lake. Within this large hydrothermal area samples of *B.*
106 *azoricus* were taken from two locations. In the active zone to the northwest, samples
107 were taken adjacent to Bairro Alto (37°17'34"N, 32°17'W). To the southeast of the lava
108 lake further samples of *B. azoricus* were collected in proximity to Eiffel Tower
109 (37°17'19"N, 32°16'29"W). Further south along the ridge from Lucky Strike is Rainbow
110 (Fig. 1). Located at 36°13'N, 33°54'W and discovered in 1997 (Fouquet et al., 1997)
111 this field is the deepest active segment of the Azores Triple Junction with water
112 depths between 2270-2320 m (mean 2300 m, Charlou et al., 2002).

113

114 Data on the physical and chemical properties of the hydrothermal fluids in the area of
115 the MAR have been provided by Fouquet et al. (1995, 1997), Charlou et al. (1997,
116 2000, 2002), Douville et al. (1997, 1999, 2002), Langmuir et al. (1997), von Damm et
117 al. (1998), Sarradin et al. (1999), Desbruyères et al. (2001). Due to the geographical

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

124

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

142

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

146 **3. Materials and Methods**

147 **3.1. Collection**

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

155

156 **3.2. Sample Cleaning**

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

173

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

180

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

191

192 **3.3. Analytical procedure**

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

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

205

206 In the absence of an analytical reference material for marine biogenic carbonate all
207 analyses performed were calibrated against an internal reference standard consisting
208 of a homogeneous fine powder of the shells of five cleaned specimens of *B. azoricus*.

209 The mean concentrations in $\mu\text{g g}^{-1}$ (\pm standard error) from 10 individual analyses of
210 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 ,
211 Cu: 0.7 ± 0.1 , Cd: 0.6 ± 0.2 .

212

213 **3.4. Statistical analysis**

214 Principal Component Analysis (PCA) was applied to the data matrix of shell weight
215 and the measured concentrations of the minor and trace elements. The coefficient of
216 correlation (r) between these variables was calculated at a level of significance of
217 99%. In order to assess the differences in metal concentrations between populations,
218 the one-way ANOVA was applied for metal concentrations fitting normal distributions.
219 Whenever metal concentrations did not fit to normal distributions Kruskal-Wallis test
220 was used. Both tests used a minimum level of significance of 99%.

221

222

223 **4. Results and Discussion**

224 **4.1. Intra-population variability**

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

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

234

235 Studies revealing the heterogeneity in chemical composition that can develop
236 amongst the shells within the population of an individual species from a single
237 location have been conducted by Babukutty & Chacko (1992), Foster & Chacko
238 (1995), Foster et al. (1997), Cravo et al. (2002, 2007). The unifying characteristic of
239 these studies is the very marked differences in chemical composition from shell to
240 shell that can develop within a single population. Marked variability in composition
241 from shell to shell is present within populations taken from pristine environments
242 (Foster & Chacko, 1995, Cravo et al., 2002) and can be significantly amplified in
243 populations taken from environments which are less pristine. This consideration is of
244 particular relevance to the data sets presented in Table 2. The shells contributing to
245 each population were taken from locations subjected to extreme environmental
246 variability with respect to both steep concentration and physico-chemical gradients.

247

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

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

259

260 **4.2. Inter-population variability**

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

269

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

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

289

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

302

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

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

320

321 In contrast to iron and manganese there was very little difference in the mean zinc
322 concentrations amongst the shells from all four sites (Fig. 4c). The only element to
323 show preferential enrichment in the shells taken from Rainbow was cadmium (Fig.
324 4d).

325

326 **4.3. General characteristics of shell chemistry of *B. azoricus* from the MAR**

327 In all a total of 51 specimens of *B. azoricus* were taken from the four contrasting sites
328 along the MAR and analyzed individually. Summary data relating to the
329 concentrations of the minor and trace metals in these 51 specimens are given in
330 Table 4. The most striking feature of the data in Table 4 is that the degree to which
331 both the minor and trace elements replace the Ca^{2+} ions in the crystal matrix of the
332 shell of *B. azoricus* is very low compared to that which can occur in shells of coastal
333 water analogues. The minor element concentrations (mean Mg and Sr concentration:
334 407 and 943 $\mu\text{g g}^{-1}$ respectively) are exceptionally low.

335

336 Magnesium concentrations in the shell of marine molluscs can vary over the range
337 90 $\mu\text{g g}^{-1}$ (Foster & Cravo 2003) to 6144 $\mu\text{g g}^{-1}$ (Afaj & Elewe 1989). In general
338 aragonitic shells have low magnesium concentrations within the range 90 to 500
339 $\mu\text{g g}^{-1}$. Magnesium concentrations are consistently lower than those of strontium, the
340 aragonitic shells being characterised by an Mg/Sr ratio of <0.5 (Foster & Cravo

341 2003). Calcitic shells exhibit much higher concentrations over a wider range and
342 concentrations above $3500 \mu\text{g g}^{-1}$ are not unusual. The magnesium concentrations
343 are generally much higher than those of strontium and the Mg/Sr ratio is generally
344 >1 . In species with bi-mineral shells the magnesium concentrations are of
345 intermediate values depending on the relative amounts of calcite and aragonite
346 contributing to the shell crystal matrix. The shells of *Mytilus* species are of mixed
347 mineralogy (Wilbur 1972, Milliman 1974, Lowenstam and Weiner 1989). Consistent
348 with the *Mytilidae* the shell of *B. azoricus* is also of mixed mineralogy (Kádár & Costa
349 2006). To date however there are no quantitative data on the relative contributions of
350 the calcite and aragonite polymorphs. The magnesium concentrations are very low
351 for a shell of mixed mineralogy and is easily within the range of concentrations
352 exhibited by purely aragonitic shells.

353

354 Mineralogy plays only a secondary role in controlling strontium concentrations
355 associated with the shell of marine molluscs (Harriss, 1965) and concentrations
356 generally fall in the range 950 to $3500 \mu\text{g g}^{-1}$. Isolated examples of concentrations
357 $<950 \mu\text{g g}^{-1}$ do exist (Afaj & Elewe 1989, Foster & Cravo 2003). The low strontium
358 concentrations with values falling to as low as $536 \mu\text{g g}^{-1}$, confirms this species as
359 having one of the most strontium impoverished shells amongst the marine molluscs.

360

361 Both magnesium and strontium are major elements in seawater. The above data
362 indicates that with respect to the shells of marine molluscs in general both these
363 elements find it particularly difficult to substitute for Ca^{2+} ions in the crystal matrix of
364 the shell of *B. azoricus*. Reference to Table 4 indicates that this difficulty is not
365 unique to the minor elements. Although the shells originate from hydrothermal trace
366 metal rich environments the trace metal concentrations in the shells are also low. The
367 mean trace metal concentrations in the crystal matrix of *B. azoricus* decrease in the
368 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

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

377

378 **4.4. Non site specific trends in the shell chemistry of *B. azoricus* from the MAR**

379 The inter-population comparison of shell composition showed that differences in
380 ambient seawater chemistry imparted site specific chemical fingerprints to the crystal
381 matrix with respect to the concentrations of iron, manganese and cadmium (Fig. 4).
382 Ambient seawater chemistry is however only one of many factors that can influence
383 the chemical composition of the shell. For a particular species parameters such as
384 size, age, sex, stage of growth, rate of growth and diet may all variously influence the
385 composition of shell. As a first approximation it has proved useful to equate the single
386 parameter of shell weight as synonymous with the multitude of agencies that may
387 variously influence the growth of the shell (Foster & Chacko, 1995; Cravo et al.,
388 2002).

389

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

396

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

410

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

420

421 The shell of *B. azoricus* is composed of both calcite and aragonite (Kádár & Costa
422 2006). As such it is most likely that the changes in the Mg/Sr ratio during shell growth
423 are induced by subtle changes in the relative amounts of calcite and aragonite
424 contributing to the crystal matrix. That the Mg/Sr ratio decreases (i.e. magnesium

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

428

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

436

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

446

447

448 **5. Conclusions**

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

453 contributing to the mineralogy of the shell. Such considerations lead Cravo et al.
454 (2007) to infer that inter-population differences in shell composition induced by
455 differences in ambient seawater chemistries may be swamped and lost amongst the
456 gross heterogeneities in shell chemistry induced by other factors. The preceding
457 data, relating specifically to the shells of *B. azoricus* within the weight range 0.62 to
458 15.70 g and to the chemistries of the waters contiguous to the Menez Gwen, Eiffel
459 Tower, Bairro Alto and Rainbow vents, substantiate this inference with respect to
460 magnesium, strontium, copper and zinc. In contrast, site-specific chemical
461 fingerprints are imparted to the crystal matrix with respect to the concentrations of
462 iron, manganese and cadmium. The inference is clearly not upheld with respect to
463 these three elements.

464

465

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707 Figure. 3. Shell weight (g) and magnesium (a), strontium and iron (b), manganese
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713 Eiffel Tower (ET), Bairro Alto (BA) and Rainbow (R).

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717 the Mg/Sr ratio, c) copper concentration and d) zinc concentration in the shell of *B.*
718 *azoricus* from the Mid-Atlantic Ridge (n=51).

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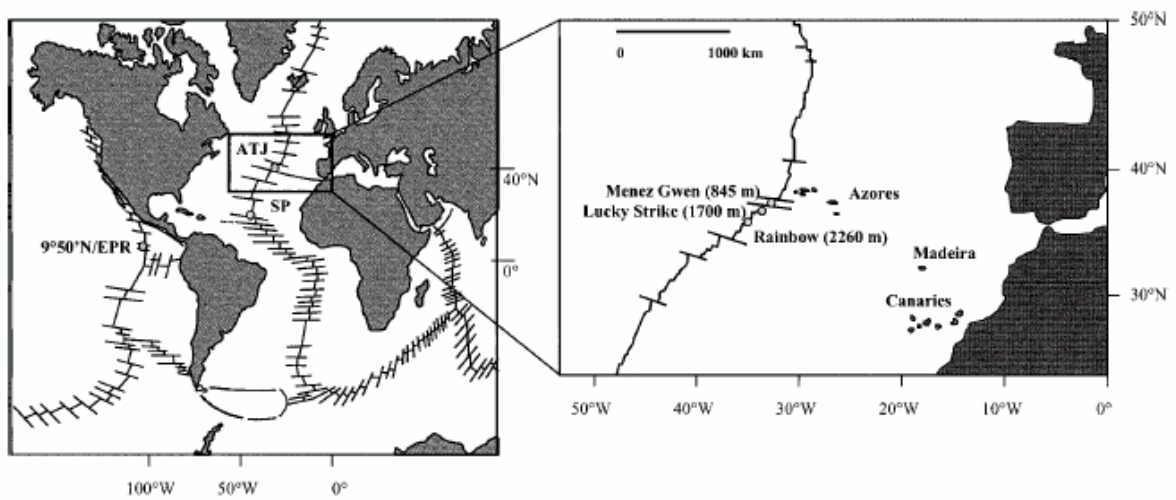
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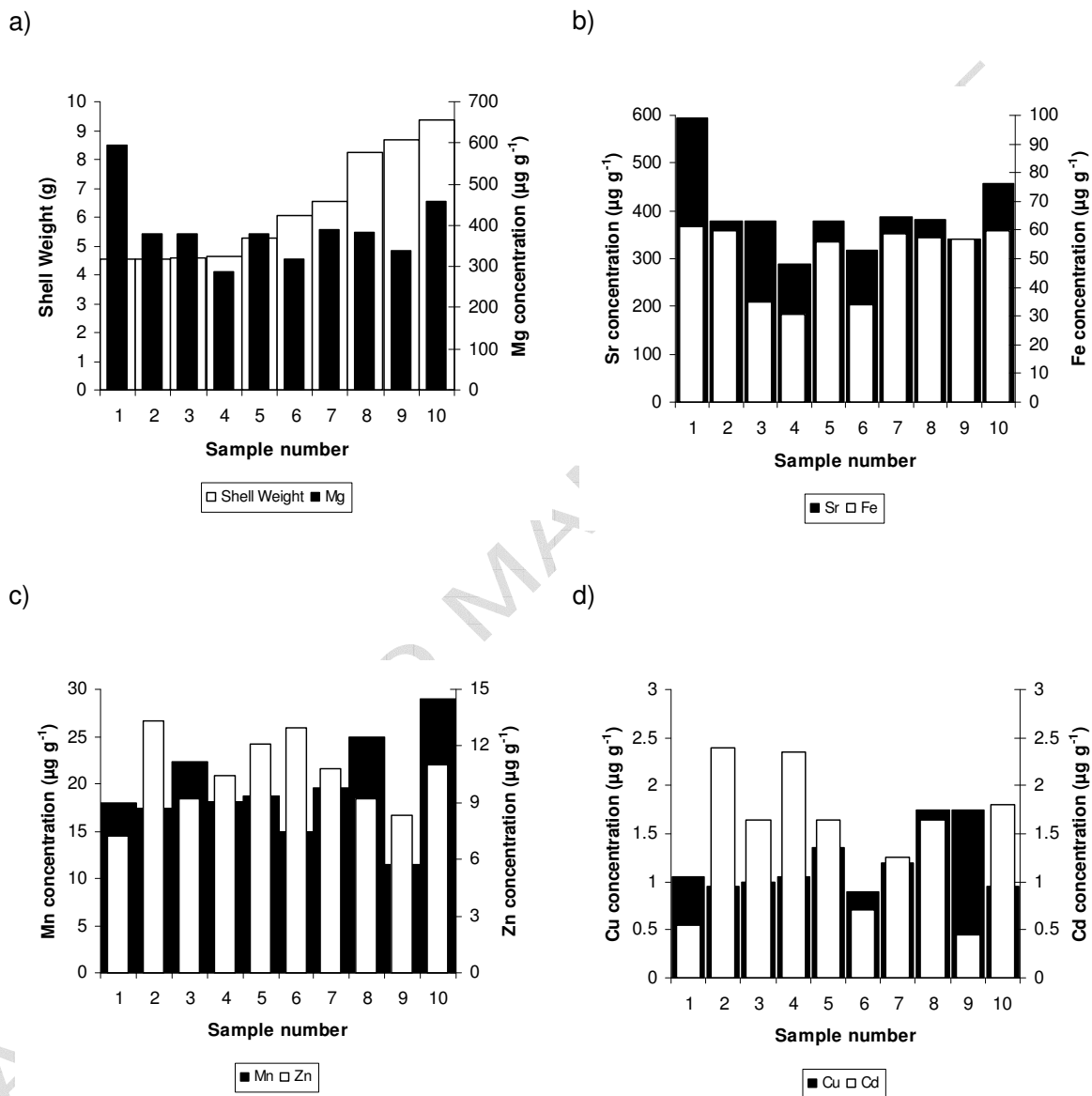
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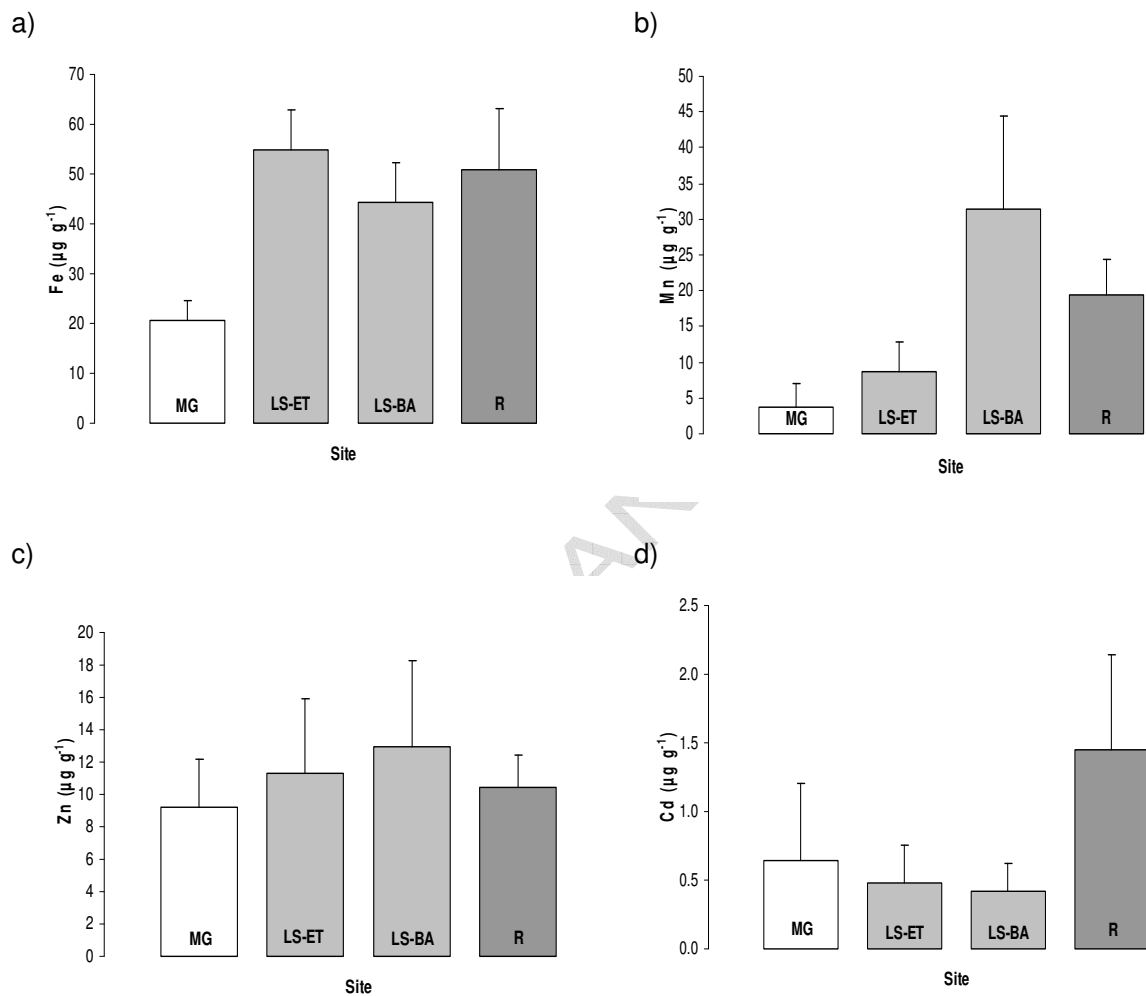


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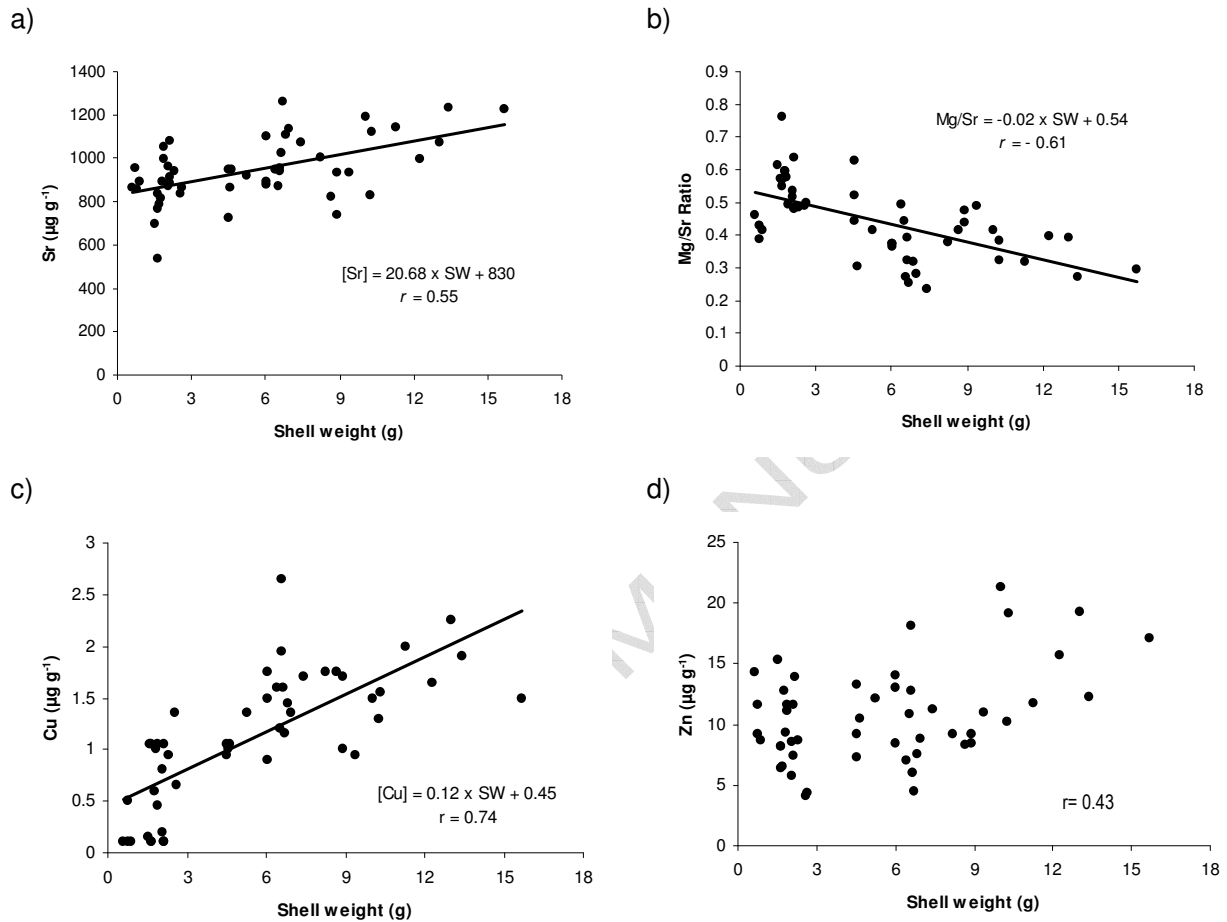
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766 the Mg/Sr ratio, c) copper concentration and d) zinc concentration in the shell of *B.*
767 *azoricus* from the Mid-Atlantic Ridge (n=51).
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772 Table 1. Comparative data on the physical and chemical 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
 777

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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