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Relationship between metal levels in the vent mussel *Bathymodiolus azoricus* and local microhabitat chemical characteristics of Eiffel Tower (Lucky Strike)

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Abstract:

The turbulent mixing of hydrothermal hot fluid with cold seawater creates large chemical gradients at a small spatial scale that may induce variable physiological and biochemical adaptations within the vent fauna. The adaptation to such a variable environment by the vent mussel *Bathymodiolus azoricus* relies on a dual symbiosis hosted in the gills, and digestion of particulate organic matter. The surrounding environment not only provides the necessary energy sources and suspended organic particles for the vent mussel nutrition, but also potentially toxic compounds such as metals. Our main goal was to see if there is a relation between metal accumulation in mussel organs and the chemical characteristics of their close environment. Mussels were collected at six locations in a cold part of the Eiffel Tower fluid-seawater mixing zone, characterized by distinct chemical compositions. Metals (Cd, Cu, Fe and Zn) and metallothioneins were quantified in the gills and digestive gland. The physiological condition of the sampled mussels was also evaluated using tissues and gill indices. Our study indicates that the accumulation of metals in *B. azoricus* is related to their spatial distribution and linked to fine scale environmental conditions that influence the physiological status of the organism.

Research highlights

Bathymodiolus azoricus were collected along a hydrothermal chemical gradient. Metals and metallothioneins were quantified in the gills and digestive gland. Metal levels reflect mussels spatial distribution and physiological status. Metallothionein levels are high and almost constant.

Keywords: Metals; Metallothioneins; *Bathymodiolus azoricus*; Hydrothermal vent; Environmental conditions; Physiological condition; Spatial distribution

1. Introduction

Deep-sea hydrothermal vents are unpredictable habitats characterized by heterogeneous venting. Turbulent mixing of hot fluids with cold seawater creates large

gradients in the environmental conditions (Tunnicliffe 1991; Childress et al. 1992; Le Bris et al. 2001) that may induce small scale variability in physiological and morphological features from one individual to the other (Tunnicliffe 1991). Therefore, it is generally assumed that abiotic factors, such as fluid flow, temperature and chemical composition, play a major role in structuring vent communities (Sarradin et al. 1999; Sarrazin et al. 1999; Cuvelier et al. 2009). Investigating the interactions between vent fauna and their environment is an essential step to understand the adaptation of species to such stochastic ecosystems.

The vent mussel *Bathymodiolus azoricus* is the dominant megafaunal species at Lucky Strike hydrothermal field (Desbruyères et al. 2001) and the main constituent of Eiffel Tower edifice assemblages (Cuvelier et al. 2009). The main source of energy of *B. azoricus* is provided by thiotrophic and methanotrophic bacteria living in symbiosis in their gills (Fiala-Médioni et al. 2002; Duperron et al. 2006; Riou et al. 2008). In this association both partners have nutritional benefits. While the endosymbionts fix inorganic carbon (e.g. CO_2 and CH_4) into organic matter using chemical energy from the oxidation of reduced sulfur and methane compounds, the host mussel facilitates their access to the essential substrates (e.g. O_2, CO_2 and reduced compounds) (Stewart et al. 2005). Nevertheless, the existence of a functional gut (Le Pennec et al. 1990) suggests that *B. azoricus* may use suspension-feeding as a secondary pathway of nutrition (Martins et al. 2008; Colaço et al. 2009; De Busserolles et al. 2009; Riou et al. 2010). The ability of the mussel to filter not only provides the necessary particulate material and substrates to fulfill the heterotrophic and chemoautotrophic processes (Le Pennec et al. 1990) but may also increase metal uptake by exposing the tissues to metal-rich vent fluids (Charlou et al.

62 2000; Douville et al. 2002; Kádár et al. 2005b). Therefore, the distribution of the vent
63 mussels along the chemical gradient can also be linked to their ability to sustain highly
64 variable metallic bioavailable forms.

65 The behavior and speciation of metallic complexes have an important biological
66 significance because they may strongly affect the uptake process of the organism
67 (Simkiss 1998). Consequently, metal bioaccumulation in vent mussels reflects partly the
68 abundance and chemical specificity of the metal in the environment surrounding the
69 mussels and also individual physiological functioning (Cosson et al. 2008).
70 Metallothioneins (MTs) are low molecular weight metalloproteins with a high cysteine
71 content, a non-enzymatic nature and a strong affinity for metal cations (Cd, Cu, Zn)
72 enabling MTs to be distinguished from most other proteins (Amiard et al. 2006). MT
73 induction in bivalve mollusks is often presumed to occur as a result of exposure to metals
74 in contaminated environments. The role of MTs in metal intracellular regulation and
75 detoxification is well established (Langston et al. 1989; Viarengo et al. 1993; Geret et al.
76 1998; Cosson 2000; Hamza-Chaffai et al. 2000; Hardivillier et al. 2006; Company et al.
77 2010). Metallothioneins protect cells against damages induced by oxidative stress (Sato et
78 al. 1993; Viarengo et al. 2000) and may be considered both as biomarkers of metal
79 exposure and physiological stress in general (Cosson 2000; Kondoh et al. 2003).
80 Metal regulation and storage in *B. azoricus* tissues have been studied by several authors
81 (Cosson et al. 1995; Martins et al. 2001; Company et al. 2004; Kádár et al. 2005b; Colaço
82 et al. 2006; Cosson et al. 2008; Martins et al. 2009). However, the link between fine scale
83 environmental variations and metal accumulation is still unknown. Here, we describe
84 levels of essential (Cu, Fe and Zn) and non essential (Cd) metals in gills and digestive

gland of *B. azoricus* individuals, collected along the cold part of the Eiffel Tower fluid-seawater mixing zone. Our main goal was to investigate the relation between the amount of metals accumulated in mussel organs and the chemical variations of their immediate environment that may influence their physiological condition. The study provides novel and important information regarding the adaptation of *B. azoricus* to extreme vent environments, in particular the ability of this species to manage the small scale environment fluctuations and the consequent supply of metals in dissolved and particulate forms.

2. Material and Methods

2.1. Study area

The Lucky Strike (LS) hydrothermal vent field (37° 18' N, 32° 16' W) extends over 1 km^2 on the summit of a prominent volcano, at the center of the LS segment of the Mid-Atlantic Ridge (MAR) (Ondréas et al. 2009). The LS field consists on three seamounts distributed around a large lava lake (Fig. 1) at depths varying from 1650 to 1750 m (Fouquet et al. 1995; Ondréas et al. 2009). Site to site variations in hydrothermal fluid temperatures (170 to 324 ºC), as well as gas and metal concentrations, suggest the presence of two fluid sources (Charlou et al. 2000). Eiffel Tower is a well-defined hydrothermal edifice and one of the most active at LS. Located in the south-eastern region of the vent field, Eiffel Tower extends 11 m in height and 20 m in width. The work developed by Cuvelier et al. (2009) divided the edifice structure into two parts, a 'tower' structure with the summit at 1681 m depth and a 'periphery' that is located on the seafloor at depths varying from 1688 to 1692 m. *Bathymodiolus azoricus* is the dominant

megafaunal species that covers the edifice walls of Eiffel Tower, being mainly distributed on the west edges of the edifice (Desbruyères et al. 2001; Cuvelier et al. 2009).

2.2. Sampling

The MoMARETO cruise (Sarrazin et al. 2006) was held in August 2006 on the French R/V "Pourquoi Pas?" with the Remotely Operated Vehicle (ROV) "Victor 6000". During this cruise studies were focused on the spatial and temporal dynamics of hydrothermal communities colonizing the MoMAR zone, centering most of the dives on LS hydrothermal field. Fauna and chemical data were collected on the 11 m high Eiffel Tower on a total of twelve locations (C1-C12) that were considered to be representative of *B. azoricus* distribution around the sulfide edifice. The results are reported in more detail in various publications (Cuvelier et al. 2009; De Busserolles et al. 2009; Sarradin et al. 2009). For the present study, six locations (C1, C3, C4, C5, C10 and C12) were chosen for sampling (Fig. 1). Mussel assemblages were collected using the ROV "Victor 6000" arm grab. On board, the collected mussels were rinsed in sea water and the length, width and height of the shells recorded. The gills and digestive gland were dissected and preserved at -20 ºC until freeze-drying and analysis of metals and MTs contents. The chemical conditions within the mussel assemblages were measured. Tracers of fluid dilution (temperature and pH), energy source (total dissolved sulfide, TdS) and potential bioavailable metal sources (total dissolved copper, TdCu, and total dissolved iron, TdFe) were analyzed at the scale of the animals at the 6 sampling locations (2-5 water samples per location). Technical limitations did not allow the in situ measurement of methane (CH_4). Water sampling and preservation are described in detail elsewhere (Sarradin et al.

2009). Temperature was measured with an autonomous temperature probe (NKE) attached to the sampling inlets. The pH measurements were performed on board at 25 ºC using a Metrohm® pH-meter with a combined pH electrode (Ingold®) for sulfide-rich medium (± 0.01 precision) after calibration with NBS buffers (pH 4 and 7). TdCu was measured in the laboratory by Stripping ChronoPotentiometry (SCP) with a gold electrode (Riso et al. 1997). TdS and TdFe were measured using the CHEMINI in situ analyzer (Vuillemin et al. 2009).

2.3. Samples preparation

Approximately 100 mg of lyophilized and grounded tissues were homogenized in 6 ml of ice-cold 100 mM Tris buffer, pH 8.1, containing 10 mM ß-mercaptoethanol. The homogenates were centrifuged for 30 min at 25 000 g, at 4 °C and an aliquot (1 ml) of the supernatant was used for metallothionein determination. The remaining supernatants and the pellets were digested simultaneously with nitric acid (65% v/v, p.a.) at 60 ºC for metal analysis. After digestion, solutions were dried at 60 ºC and the resulting material was solubilized with 2 ml of 0.5 N HNO_3.

2.3.1. Metal analyses

Copper, Fe and Zn were determined by flame atomic absorption spectrophotometry (GBC-Avanta Σ), with deuterium background correction. Cadmium was determined by graphite furnace atomic absorption spectrometry (Perkin–Elmer, Zeeman 4110ZL). The accuracy and precision of the method used were established by regular analysis of certified reference materials, mussel tissue CE278 (European

154 Reference Materials of Belgium) and lobster hepatopancreas TORT-2 (National Research
155 Council of Canada). Certified reference materials and blanks were taken through the
156 procedure in the same way as the samples. Measured values and certified values are given
157 in Table 1 as μg g^{-1} of dry weight. The Zn levels measured in the certified material
158 presented overestimated values. Metal levels in mussel whole tissues were also
159 expressed as μg g^{-1} of dry weight.
160
161 *2.3.2. Metallothionein analysis*
162 The 1 ml aliquot of the supernatant, obtained from the centrifugation described in
163 section 2.3, was heat-denatured (90 °C, 15 min) and centrifuged for 10 min at 13 000 g,
164 at 4ºC, in order to separate the heat stable metallothionein (MT) from thermo labile
165 compounds. The heat stable fractions were used for the quantification of MT by
166 Differential Pulse Polarography (DPP) according to Olafson and Sim (1979) and
167 Thompson and Cosson (1984). A standard addition calibration curve was obtained using
168 rabbit liver MT-I as reference. Results were expressed as μg g^{-1} of dry weight.
169
170 *2.4. Condition indices*
171 The tissue condition index (TCI) and gill index (GI) were used to assess the
172 physiological condition of the collected mussels. The tissue condition index was
173 determined according to Voets et al. (2006): TCI = tissues dry weight (g)/mussel shell
174 volume (ml). The mussel shell volume (V_m) was calculated based on the length, width
175 and height of the mussel shell with the formula: V_m = (length x width x height)/C. C is a
176 constant determined empirically as follows. The volume of the space enclosed by the

177 shell valves (V_m) of 132 *B. azoricus* individuals between 24 and 93 mm lengthy was
178 measured to the nearest ml by displacement of water in a graduated cylinder. Empty
179 shells were sealed with parafilm before immersion in the liquid. Values of V_m were then
180 plotted against the volume of the cube (V_{cube}) obtained by multiplying the length x width
181 x height. The regression line was calculated and the slope (constant C) amounted to 2.6 ±
182 0.03 (R^2= 0.988, $p<0.05$) (unpublished data). The gill index was calculated as follows: GI
183 = (gill tissue dry weight (g)/shell volume (ml)) x 10.
184
185 *2.5. Data analysis*
186 All data were tested for normality by normal probability plots and homogeneity of
187 variances by Bartlett's test. Since data did not respect the former assumptions of
188 parametric analysis, non-parametric tests were applied. Mann-Whitney U test (M-W) and
189 Kruskal-Wallis ANOVA by ranks (K-W) were used to evaluate the variability between
190 the different groups of samples. Dunn's test was used as post hoc comparison of means.
191 Principal Component Analysis (PCA) was used to investigate the spatial patterns of the
192 relative levels of analyzed metals and metallothioneins within the individuals of the 6
193 locations. Data was standardized before PCA analysis. The tests were performed with
194 STATISTICA 6.0 (StatSoft). Differences were considered significant when $p<0.05$.
195 Statistical methods were selected in accordance with Zar (1999).
196
197 **3. Results**
198 *3.1. Environmental conditions*

199 Mean values of environmental chemical parameters at each of the 6 sampled
200 locations were extracted from De Busserolles et al. (2009) and summarized in Table 2.
201 Mean temperatures and pH varied from 4.8 to 8.8 ºC and 6.0 to 7.1 respectively. The
202 location with the warmest temperature value (C10) also had the highest concentrations of
203 TdS and TdFe and the lowest concentration of TdCu. An opposite pattern was observed
204 at the coolest location since C1, C5 and C12 had the lowest TdS and TdFe
205 concentrations. However, TdCu concentrations did not follow a similar trend.
206
207 *3.2. Mussel sizes*
208 Table 3 gives the number of mussels collected at each location and the mean shell
209 allometric parameters. The mussels from locations C3 and C10 have significantly larger
210 shell length than mussels from locations C1, C4, C5 and C12 (K-W, $p<0.05$). However,
211 mussels from C3 and C10 have significantly similar shell lengths (K-W, $p>0.05$) as do
212 the mussels from C1, C4, C5 and C12 (K-W, $p>0.05$).
213
214 *3.3. Metal and MT levels*
215 Mean metal and MT levels in the gills and digestive gland of mussels collected at
216 the 6 locations, are given in Figures 2. In brief, whatever the collection locations, Cd
217 (Fig. 2-a), Fe (Fig. 2-c) and MT (Fig. 2-e) presented higher levels in the digestive gland.
218 Copper was present preferentially in the gills (Fig. 2-b) except for mussels from the
219 coolest location (C12). Regarding Zn, no relationship was observed between gills or
220 digestive gland levels and locations (Fig. 2-d).
221

222 *3.3.1. Metal organotropism between locations*

223 Cadmium levels were statistically higher in the digestive gland than in the gills
224 (M-W, $p<0.05$) for mussels from the locations C3, C4 and C5. On the other hand, Cu
225 levels were statistically higher in the gills than in the digestive gland (M-W, $p<0.05$),
226 except for C12, where Cu levels were higher in the digestive gland (M-W, $p<0.05$). No
227 significant difference was observed between Cd levels of both tissues (M-W, $p>0.05$) in
228 mussels from the warmest location (C10) or from the coolest one (C12). At the 6 sampled
229 locations, Fe and MT levels were higher in the digestive gland than in the gills (M-W,
230 $p<0.05$). At the location C1 and C10, gills showed higher levels of Zn than the digestive
231 gland (M-W, $p<0.05$), the opposite was found in mussels from location C5. No
232 statistically significant differences were found for Zn levels between both tissues (M-W,
233 $p>0.05$) in mussels from locations C3, C4 and C12.

234

235 *3.3.2. Metal levels between locations*

236 The gills of mussels from locations C1, C3, C4 and C10 showed similar levels of
237 Cd (K-W, $p>0.05$), lower than those observed for locations C5 and C12 (K-W, $p<0.05$),
238 which were not significantly different (K-W, $p>0.05$). The digestive glands of mussels
239 from the locations C1, C4, C10 and C12 showed similar levels of Cd (K-W, $p>0.05$) as
240 did mussels from locations C3 and C5 (K-W, $p>0.05$). The gills of mussels from the 6
241 different locations showed similar levels of Cu (K-W, $p>0.05$) with the exception of
242 those from C4, which had the lowest values (K-W, $p<0.05$). The digestive glands of
243 mussels from C1, C4, and C3, C5, C10 showed similar levels of Cu, respectively (K-W,
244 $p>0.05$). The gills of mussels from the different locations showed similar levels of Fe (K-

245 W, p>0.05) with the exception of those from locations C5, which had the lowest values
246 (K-W, p<0.05). The digestive glands of mussels from the different locations showed
247 similar levels of Fe (K-W, p>0.05) with the exception of those from the locations C4,
248 which exhibited the highest values (K-W, p<0.05). However, concentrations at C4 were
249 not significantly different from those at C5 (K-W, p>0.05). The gills of mussels from the
250 locations C1, C3, C4, C5 and locations C10 and C12 showed similar levels of Zn (K-W,
251 p>0.05). The lowest mean level was observed at C4 while the highest values were
252 measured at C10 and C12. The digestive glands of mussels from locations C1, C3, C4
253 and C10 showed similar levels of Zn (K-W, p>0.05),. The highest mean level of Zn was
254 observed at C12. No difference could be established between MT levels in both organs of
255 mussels with the exception of mussels from location C12. Here, the gills had higher
256 levels of MT than the gills of mussels from C4 and C10 (K-W, p<0.05), while the
257 digestive glands had higher levels of MT than the digestive glands of mussels from C1
258 (K-W, p<0.05).
259
260 *3.3.3. PCA*
261 A principal component analysis (PCA) was used to investigate the spatial
262 distribution of mussels relative levels of metals and MT in both organs, and shell length,
263 over the individuals. For each tissue, PCA clearly separated individuals between the
264 locations according to their relative levels of the different metals and MT, and shell
265 length (Fig. 3-4). The first two principal components accounted for 56.4 % of the
266 variability in the metal levels in the gills, with 34.5% on axis 1 and 21.9% on axis 2 (Fig.
267 3A). Several groups of mussels were discriminated. Starting from the right side of axis 1

and moving towards the left, mussels from C4 were followed by those from C1, C3 and C12. This axis discriminates according to the relative levels of Cu, Zn, Cd, and MT (Fig. 3B). On the second axis, mussels from C10 are discriminated from C5 mussels with respect to the relative levels of Fe and shell length.

In the digestive gland, the PCA showed that the first two axes accounted for 60.8 % of variability between the mussels, with 38.9 % explained by axis 1 and 21.9% by axis 2 (Fig. 4A). Axis 1 discriminates according to the relative levels of Zn, Cd, Cu and MT, while axis 2 discriminates positively Fe levels and negatively the shell length (Fig. 4B). Mussels from locations C1, C3, C4 and C10 (right of axis 1) separated from C12 mussels (left of axis 1). Mussels from C10 were separated from those from other locations along axis 2.

Results of these two PCAs show that: (i) there is a clear segregation in the metal accumulation by *B. azoricus* from different sampling locations, (ii) this segregation is observed for both studied organs, (iii) the size of the mussels is also a discriminating factor.

3.3.4. TCI and GI

The mean values of tissue condition index (TCI) and gill index (GI) in mussels collected at the 6 locations are shown in Fig. 5. In order to limit the effect of mussel size, comparisons were made among mussels from locations C1, C4, C5 and C12 (mean length < 6 cm) and among mussels from locations C3 and C10 (mean length > 6 cm). Therefore, we observed that mussels from location C1 and C4 showed the highest mean TCI and GI

290 (K-W, $p<0.05$). The TCI in mussels from location C10 was higher than in mussels from
291 location C3 (M-W, $p<0.05$), although their GI were not different (M-W, $p>0.05$).
292
293 **4. Discussion**
294 The Eiffel Tower is an active edifice in the LS vent field largely colonized by *B.*
295 *azoricus* faunal assemblages (Desbruyères et al. 2001; Cuvelier et al. 2009; Sarradin et al.
296 2009). The mean temperatures varied from 4.8 to 8.8 ºC. This is a relative narrow range
297 for vent ecosystems (De Busserolles et al. 2009), although it is characteristic of the cold
298 part of the mixing zone where a low percentage of hot hydrothermal fluids (T= 324 ºC,
299 pH 3.5-4.2) mixes with cold seawater (T= 4.4 ºC, pH 7.8) (Sarradin et al. 2009). Such a
300 mixing zone is subject to short temporal (seconds) and spatial (centimeters) gradients of
301 the physicochemical conditions, which may critically affect the concentrations of the
302 substrates used as energy and carbon sources for chemosynthetic processes (Le Bris et al.
303 2003; Stewart et al. 2005). Consequently we can hypothesize that local environmental
304 variations influence the physiological status of the vent mussels. Temperature has a semi-
305 conservative behavior and is affected by physical processes occurring at vents. At known
306 sites it can be used as a tracer of hydrothermal fluids (Sarradin et al. 2008; Sarradin et al.
307 2009). Moreover, De Busserolles et al. (2009) demonstrated that, at Eiffel Tower,
308 temperature is positively correlated with dissolved sulfide and iron concentrations and
309 negatively correlated with dissolved Cu. However, it seems that both dissolved and
310 particulate forms of metals are not controlled by a simple dilution process, as shown by
311 Sarradin et al. (2008) in two distinct microhabitats of the East Pacific Rise (EPR)
312 hydrothermal field. Other factors that may account for metal availability and

313 accumulation in vent mussels include (i) the ability of metals (like Cd, Cu, Fe and Zn) to
314 form metallic complexes with sulfides in plume or conduit surfaces (Feely et al. 1994),
315 which precipitates and are exported in the buoyant plume settling close to the organism
316 (Trefry et al. 1985; Trocine et al. 1988), and (ii) the occurrence of metallic complexes
317 dissolution/oxidation reactions in oxygen-enrich mixing zones (Sarradin et al. 2008;
318 Sarradin et al. 2009). The PCAs performed in this study (Figs. 3-4) indicated that mussels
319 from location C12 are clearly distinct from mussels from location C4 in terms of the
320 amounts of metals present in their tissues.
321
322 *4.1. Location C12*
323 Among the sampled locations, C12 is characterized by the lowest temperature,
324 highest pH and one of the lowest concentrations of TdS. It represents an environment
325 where the hydrothermal fluid is largely diluted. In these conditions the energy supply
326 (H_2S and CH_4) is limited and metal levels are relatively low. The bioaccumulation
327 pathway of metals is not strictly governed by the concentrations of these metals in the
328 environment but is strongly influenced by the hydrophilic and hydrophobic properties of
329 the dissolved metals (Sarradin et al. 2009). The high concentrations of total dissolved Cu
330 (TdCu) measured at C12, and the high concentrations of Cu found in the mussel gills
331 from this location, seem to be related. A recent study carried out by Sarradin et al.,
332 (2009) on the same edifice showed that most of the dissolved Cu results from an
333 oxidative redissolution process. This phenomenon results not only in a secondary source
334 of dissolved Cu to the vent mussels but also in a Cu fraction more bioavailable to the
335 organisms, as most of the dissolved Cu is present in the form of inorganic or hydrophilic

336 organic complexes (Sarradin et al. 2009). Redissolution reactions could also occur with
337 other metals, resulting in their higher bioavailability for the mussels. Nevertheless, the
338 highest levels of Cu and Fe were found in the digestive gland rather than in the gills. This
339 observation may indicate that suspension-feeding is the main path for both Cu and Fe
340 uptake. In such a diluted environment, *B. azoricus* can not rely on the thiotrophic and
341 methanotrophic endosymbionts to fulfill their nutritional needs. Therefore, its ability to
342 feed on suspended organic particles (Le Pennec et al. 1984; Riou et al. 2010), including
343 metal-sulfide organic complexes (Taillefert et al. 2002), gives the vent mussel the
344 opportunity to cope with unpredictable environments but may also increase the input of
345 metals in the digestive gland.
346
347 *4.2. Locations C1, C3, C4 and C5*
348 The distribution of metals between the studied organs was similar for the mussels
349 collected at these four microhabitats. Besides the fact that mussels from location C3
350 showed a higher mean length, than those from the other locations, metal organotropism
351 seems not to be influence by size (Boyden 1974; Mubiana et al. 2006). Iron and Cd
352 accumulated preferentially in the digestive gland and Cu in the gills. Zinc distribution did
353 not show a particular pattern. In the mixing zone, Sarradin et al., (2009) found a transition
354 area between high sulfide/low oxygen waters and low sulfide/high oxygen waters. The
355 increase of sulfide and dissolved metals enhances the formation of particle metal-sulfide
356 (MS) stable complexes that are less bioavailable for mussels. The formation of such MS
357 complexes was demonstrated earlier for Cd, Cu, Fe and Zn (Luther et al. 2001; Di Meo-
358 Savoie et al. 2004; Sarradin et al. 2008) and Cu (Sarradin et al. 2009) at EPR and MAR

359 hydrothermal fields, respectively. The higher concentrations of total dissolved sulfide
360 (TdS) measured at C4 may be responsible for an increased formation of MS that would
361 explain the lower metal levels found in the gills compared to those found in the digestive
362 gland. The gills of the mussels collected at location C5 showed the lowest levels of Fe, in
363 accordance with the lowest TdFe concentrations measured at C5. Iron differs from the
364 other metals present at the hydrothermal fields in terms of both its semi-conservative
365 behavior (Luther et al. 2001; Sarradin et al. 2008), partially controlled by dilution
366 processes, and the abundant formation of FeS-metal precipitates (Johnson et al. 1988; Di
367 Meo-Savoie et al. 2004). When sulfide concentrations increase in the mixing zone, the
368 reductive dissolution of soluble organic-Fe is followed by the formation of iron molecular
369 clusters (FeS_2) (Luther et al. 2001) that react highly with other metals reducing
370 significantly the amounts of bioavailable Fe (Di Meo-Savoie et al. 2004). On the other
371 hand, high levels of Fe were found in the digestive gland of mussels from this location
372 and from C4. The ability of mussels to ingest suspended particles may be responsible for
373 the high levels of Fe observed in the digestive gland. Cadmium concentrations in LS
374 hydrothermal fluids are lower than other metal concentrations (Desbruyères et al. 2001;
375 Douville et al. 2002; Kádár et al. 2005a). However, Cd burden in tissues largely depends
376 on its physico-chemical forms in the surrounding water rather than its concentration in
377 the pure fluid. In the reactive mixing zone, Cd ions may form small particles with FeS_2,
378 which may undergo dissolution and/or oxidation reaction (Sarradin et al. 2008).
379 Cadmium has been shown to bind preferentially to ligands such as the inducible
380 metallothioneins (MT) (Bebianno et al. 1991; Cosson 2000; Geret 2000). Metals bound to
381 soluble components are more difficult to excrete. Consequently, prolonged exposure to,

382 and uptake of, Cd increase the amounts of this metal in tissues (Langston et al. 1998), as
383 observed in the studied organs of C5 mussels. Copper redissolution phenomena may
384 account for its preferential accumulation in the gills rather in the digestive gland of
385 mussels collected from locations C1, C3, C4 and C5. Zinc accumulation patterns were
386 highly variable. They may reflect the variability of Zn in each of the locations, as well as
387 variable abilities of the vent mussels to reduce Zn uptake and regulate its storage, as
388 shown previously for coastal mussels (Anandraja et al. 2002; Kondoh et al. 2003; Wang
389 et al. 2005).
390
391 *4.3. Location C10*
392 The high TdS and TdFe concentrations found at this location, and the resulting
393 formation of FeS_2, may be responsible for the observed higher levels of Fe in the
394 digestive gland compared with those found in the gills. Although TdCu concentrations
395 were low, Cu levels were higher in the gills than in the digestive gland. According to
396 Martins et al. (2008), the dominant nutritional strategy of *B. azoricus* varies with body
397 size and external conditions. Larger individuals rely more on endosymbiosis for their
398 nutritional needs, which explain their spatial distribution closer to H_2S and CH_4 sources.
399 Since trace metals can be used as electron donors and acceptors by bacteria for generating
400 energy (Di Meo-Savoie et al. 2004), the autotrophic bacterial metabolism may also play a
401 role in metal (Cu and Zn) bioaccumulation in the gills.
402
403 *4.4. MTs*

Several studies undertaken with costal mussels showed that the primary role of MTs is the homeostasis of essential metals and the prevention of non-essential metal binding to other ligands resulting in a metabolism dysfunction (Roesijadi et al. 1996). However, MT is also involved in an array of protective stress responses, such as food shortage (Cosson 2000; Viarengo et al. 2000; Kondoh et al. 2003), or oxidative damage induced by metal exposure, namely Cu (Company et al. 2008) or Cd (Geret et al. 2002; Company et al. 2010). MTs levels are almost constant in the mussel tissues collected, suggesting that these organisms may rely on this metalloproteins to cope with their fluctuating immediate environment. However, mussels from location C12 showed higher levels of MT than mussel from C4 and C10, probably due to the high levels of metals in their tissues, namely Cu, and the depleted amounts of reduced compounds essential for their nutrition through the endosymbiont primary production. Moreover, higher MT levels do not always reflect higher bioavailable metals in the environment but also can result from slower MT turnover rates that depend on the associated metals (Cu-MT or Cd-MT e.g.) (Wang et al. 2010).

4.5. TCI and GI

Since the uptake and accumulation of metals in mussels are actively controlled by physiological and biochemical processes (Mubiana et al. 2006), a better nutritional status allows the mussel to cope with metal exposure. Our results corroborate this hypothesis since the mussels with higher physiological conditions (C1 and C4) showed lower metal levels in both tissues. On the contrary, mussels from location C12 showed low TCI and high metal levels. As larger mussels rely preferably on endosymbionts for their nutrition

(Martins et al. 2008), the high TCI found in mussels from C10 may result from an easier access to reduced energy sources in a slightly warmer location. Nevertheless, mussels from locations C3 have similar GI and lower TCI than mussels from C10, which can indicate that mussels from C3 use energetic reserves from other tissues rather than the gills to fulfill their requirements.

5. Conclusions

Our study indicates that there is a significant spatial variation of metal accumulation by the vent mussel *B. azoricus* on the Eiffel Tower edifice. This variation seems to be linked to local environmental conditions that affect the physiological status of the mussels and influence their ability to cope with metal exposure. The high and almost constant levels of metallothioneins in the studied mussels may suggest a background induction for a physiological adaptation to such extreme and fluctuating environments. The vent mussel is an appropriate model for assessing the responses to the metallic load brought by venting fluids. Further studies should address the storage in tissues of metals in insoluble and/or soluble forms in order to understand how *B. azoricus* manage the metals that it takes up at a subcellular scale.

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674

Table 1

Mean values (± SD) of Cd, Cu, Fe and Zn levels, in µg g^{-1} dry weight (d.w), found in certified reference material, lobster hepatopancreas TORT-2 (NRCC-Canada) and mussel tissue CE278 (ERM-Belgium). n represents the number of samples analyzed.

Certified reference material		n	Certified µg g^{-1} (d.w)	Observed µg g^{-1} (d.w)
TORT-2	Cd	21	26.7 ± 0.6	25.6 ± 1.2
	Cu	9	106 ± 10	105 ± 3
	Fe	12	105 ± 13	106 ± 6
CE278	Zn	11	83.1 ± 1.7	110 ± 8

Table 2

Mean values (± SD) of environmental conditions (temperature (T), pH, total dissolved sulfide (TdS), total dissolved iron (TdFe) and total dissolved copper (TdCu)) in each of the 6 sampled locations. Results extracted from the article of De Busserolles et al. (2009). n= 2 to 5 water samples per location.

Location	T (ºC)	pH	TdS (µmol l^{-1})	TdFe (µmol l^{-1})	TdCu (µmol l^{-1})
C1	4.9 ± 0.5	6.9 ± 0.2	1.3 ± 0.8	0.4 ± 0.4	1.6 ± 0.9
C3	5.4 ± 0.5	6.7 ± 0.2	3.4 ± 1.8	0.4 ± 0.2	1.4 ± 0.2
C4	5.7 ± 0.5	6.6 ± 0.1	6.1 ± 4.4	1.7 ± 0.8	2.1 ± 1.3
C5	5.1 ± 0.5	6.9 ± 0.1	1.9 ± 0.9	0.1 ± 0.2	0.8 ± 0.4
C10	8.8 ± 2.7	6.0 ± 0.4	34.9 ± 22.0	5.3 ± 3.6	0.5 ± 0.7
C12	4.8 ± 0.3	7.1 ± 0.6	2.3 ± 1.1	0.4 ± 0.4	2.6 ± 2.2

Table 3

Number (n) and shell size (± SD) of the mussels collected in each of the 6 sampled locations.

Location	n	Length (cm)	Width (cm)	Height (cm)
C1	20	5.1 ± 0.8	2.4 ± 0.3	1.9 ± 0.3
C3	20	6.1± 0.7	3.0 ± 0.3	2.2 ± 0.3
C4	15	5.2 ± 1.0	2.7 ± 0.3	2.0 ± 0.3
C5	20	5.2 ± 0.8	2.6 ± 0.3	1.9 ± 0.3
C10	10	7.0 ± 0.7	3.4 ± 0.3	2.7 ± 0.3

C12	20	5.4 ± 0.9	2.7 ± 0.5	2.0 ± 0.4

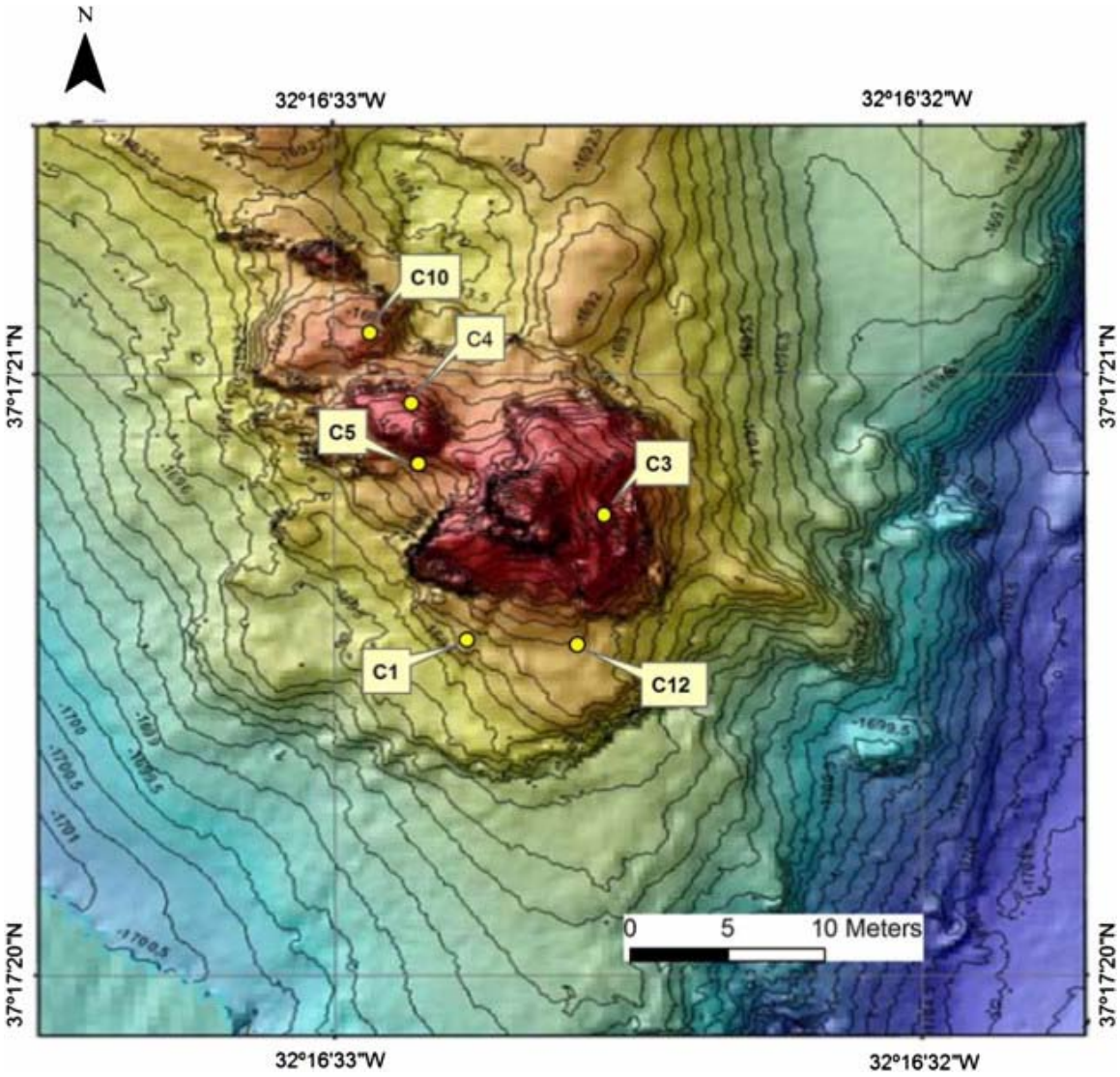

Fig. 1. Bathymetric map of Eiffel Tower active edifice, located in Lucky Strike vent field, and position of the 6 sampled locations: C1, C3, C4, C5, C10 and C12. Adapted from Sarrazin et al. (2006).

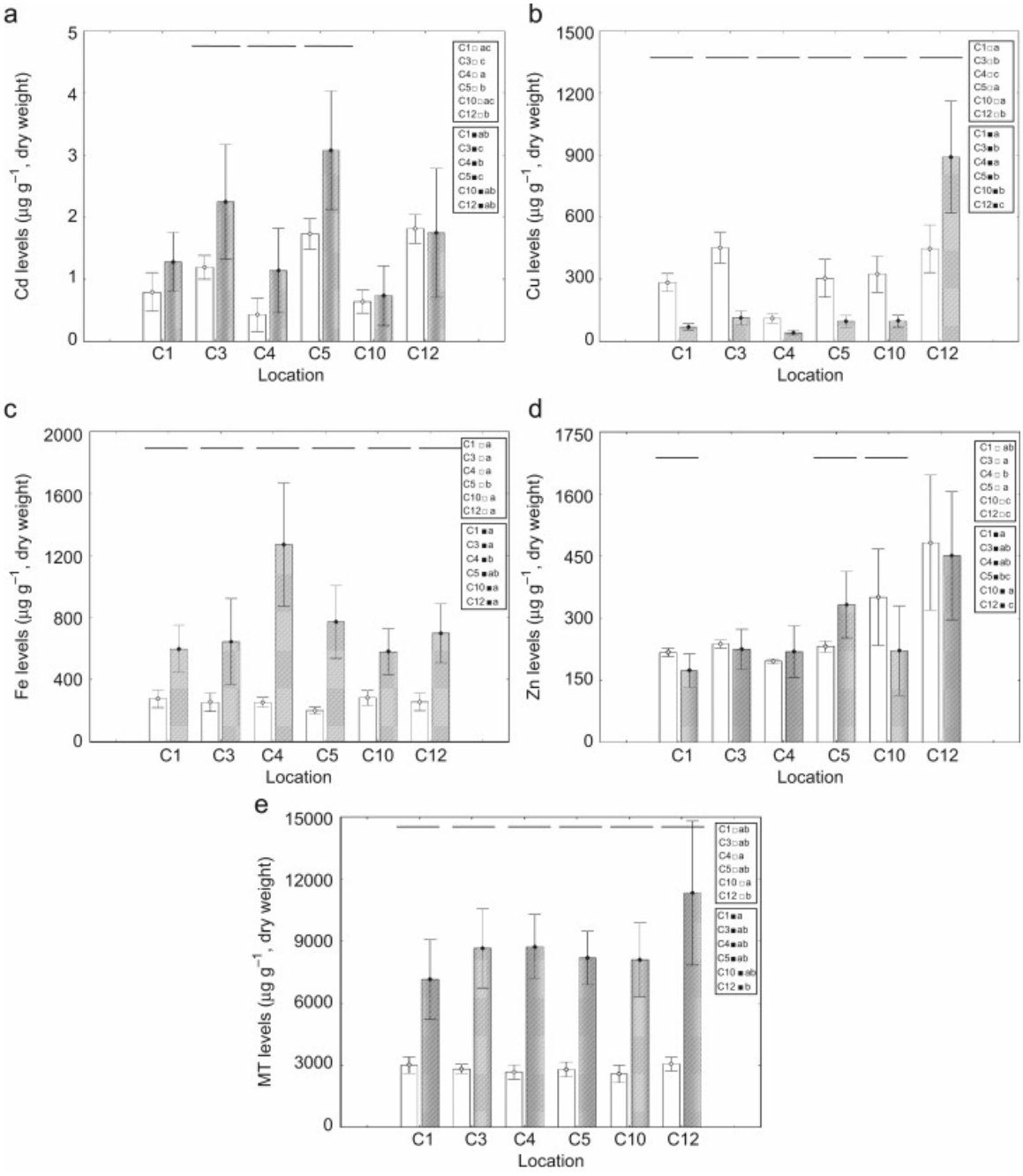

Fig. 2. Mean levels (µg g−1, dry weight) of Cd (a), Cu (b), Fe (c), Zn (d) and MT (e) in gills (open columns) and digestive gland (shade columns) of mussels collected at each location (C1, C3, C4, C5, C10 and C12). Vertical bars represent the standard deviation of the mean. Symbol (-) represents significant differences between tissues for each location. Similar letters indicate no statistical difference among locations for gills (□) and for digestive gland (■).

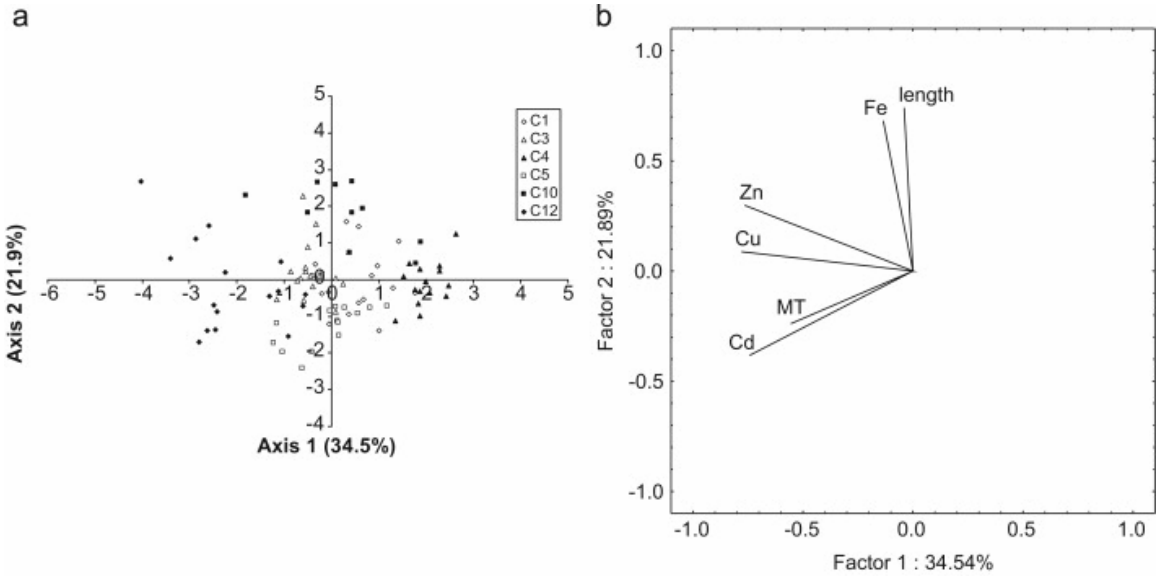

Fig. 3. First plane principal component analysis (PCA) of mussel gill metal levels at each location (C1, C3, C4, C5, C10 and C12). (a) Individual scores. (b) Descriptor scores. All variables were standardized before analysis.

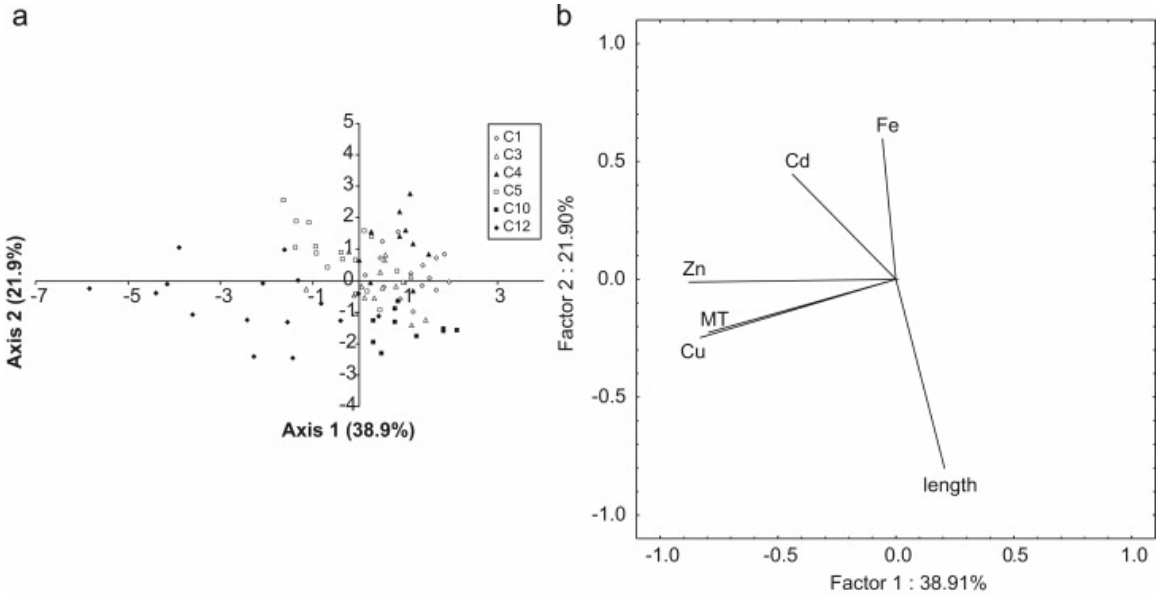

Fig. 4. First plane principal component analysis (PCA) of mussel digestive gland metal levels at each microhabitat (C1, C3, C4, C5, C10 and C12). (a) Individual scores. (b) Descriptor scores. All variables were standardized before analysis.

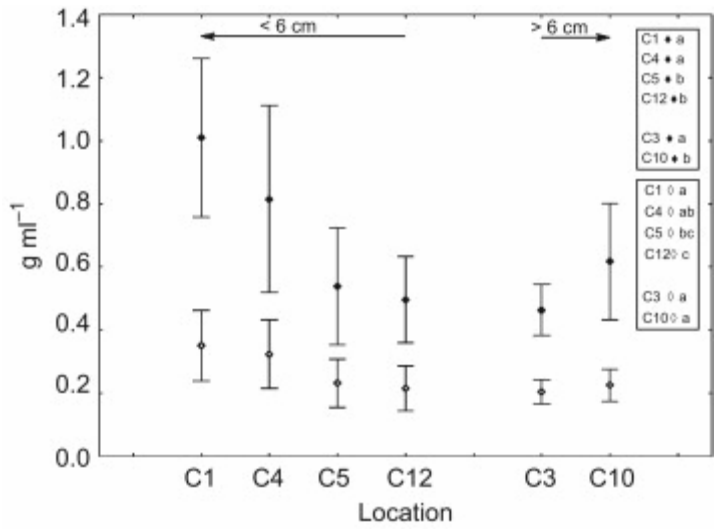

Fig. 5.

Mean (g ml^{-1}) TCI (closed diamonds) and GI (open diamonds) in mussels collected at each location (C1, C4, C5, C12 and C3, C10). Locations are separated by mussels shell length (<6 cm>). Vertical bars represent the standard deviation of the mean. Similar regular letters indicate no statistical difference among locations with mussels length <6 cm ( ) and similar italic letters indicate no statistical difference among locations with mussels length >6 cm ( ).