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

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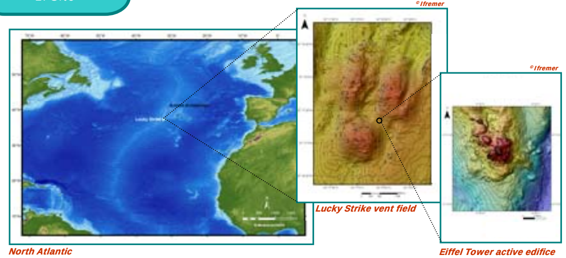
1. Introduction

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 [1,2] that may induce small scale variability in physiological and morphological features from one individual to the other [1].

The vent mussel *Bathymodiolus azoricus* is the dominant megafaunal species at Lucky Strike hydrothermal field and the main constituent of Eiffel Tower edifice assemblages [3]. The main source of energy of *B. azoricus* is provided by thiotrophic and methanotrophic bacteria living in symbiosis in their gills. Nevertheless, the existence of a functional gut suggests that *B. azoricus* may use suspension-feeding as a secondary pathway of nutrition [4]. 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 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

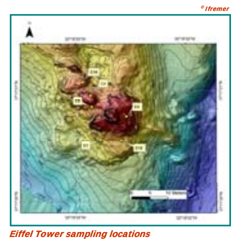
2. Site



3. Methods

Six locations (C1, C3, C4, C5, C10 and C12) were chosen for sampling that were considered to be representative of *B. azoricus* distribution around the sulfide edifice. 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 (Table 1). Mussel assemblages were collected using the ROV "Victor 6000" arm grab.

On board, the length, width and height of the shells were recorded. The gills and digestive gland were dissected and preserved at -20 ºC until freeze-drying and analysis of metals (Cd, Cu, Fe and Zn, Atomic Absorption Spectrophotometry) and metallothioneins (MT) (Differential Pulse Polarography) contents.



4. Results

4.1. Chemical Conditions

	C12	C1	C5	C3	C4	C10
T (°C)	4.8	4.9	5.1	5.4	5.7	8.8
pH	7.1	6.9	6.9	6.7	6.6	6.0
TdS (µM)	2.3	1.3	1.9	3.4	6.1	34.9
TdFe (µM)	0.4	0.4	0.1	0.4	1.7	5.3
TdCu (µM)	2.6	1.6	0.8	1.4	2.1	0.5

Table 1- Mean values of environmental conditions in each of the 6 sampled locations[5]. n= 2 to 5 water samples per location.

Mean temperatures varied from 4.8 to 8.8 ºC. The pH varied from 6.0 to 7.1 reaching more acidic values at warmer locations. The location with the warmest temperature value, C10, also had the highest concentrations of TdS and TdFe and the lowest concentration of TdCu. An opposite pattern was observed at the coolest locations since C12, C1 and C5 had the lowest TdS and TdFe concentrations. However, TdCu concentrations did not follow a similar trend.

4.2. Condition Indices

The tissue condition index (TCI) and gill index (GI) were used to assess the physiological condition of the collected mussels. The condition indices were determined as follows:

$$TCI^{1} = \frac{\text{tissues dry weight (g)}}{\text{mussel shell volume (ml)}} \qquad GI = \frac{\text{gill tissue dry weight (g)}}{\text{shell volume (ml)) x 10}}$$

Mussels mean length < 6 cm

Mussels from warmer locations, C1 and C4, showed the highest mean TCI and GI (K-W, p<0.05).

Mussels mean length > 6 cm

Mussels from the warmer location C10, showed the highest mean TCI (M-W, p<0.05). However, their GI were not significantly different (M-W, p>0.05).

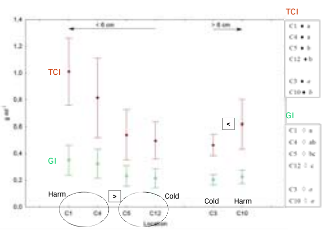


Fig. 1- Mean TCI and GI in mussels collected at each location. Locations are separated by mussels shell length (< 6 cm >). Vertical bars stand for SD. Similar letters indicate no statistical difference (K-W, p< 0,05) among locations

4.3. Metallothioneins

Comparing tissues, whatever the collection locations, MT presented higher levels in the digestive gland than in the gills (M-W, p<0,05). Comparing locations (Fig. 2), no difference could be established between MT levels in both organs of mussels with the exception of mussels from location C12. At this location, the gills had higher levels of MT than the gills of mussels from C4 and C10 (K-W, p<0.05), while the digestive glands had higher levels of MT than the digestive glands of mussels from C1 (K-W, p<0.05).

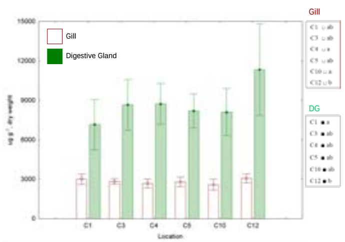


Fig. 2- Mean levels (µg g-1, dry weight) of MT in gills (open columns) and digestive gland (shade columns) of mussels collected at each location (C1, C3, C4, C5, C10 and C12). Vertical bars stand for SD. Similar letters indicate no statistical difference (K-W, p< 0,05) among locations

4.4. Metals

Comparing tissues, whatever the collection locations Cd and Fe presented higher levels in the digestive gland than in the gills (M-W, p<0,05). Copper was present preferentially in the gills (M-W, p<0,05) except for mussels from the coolest location, C12 (M-W, p>0,05). Regarding Zn, no relationship was observed between gills or digestive gland levels and locations (M-W, p>0,05).

A principal component analysis (PCA) was used to investigate the spatial distribution of mussels relative levels of metals and MT in both organs, and shell length, over the individuals. For each tissue, PCA clearly separated individuals between the locations according to their relative levels of the different metals, MT and shell length (Fig. 3-4).

Gill

Axis 1 discriminates mussels from location C12 and C4 (Fig. 3A) according to the relative levels of Cd, Cu, Zn, and MT (Fig. 3B) while axis 2 discriminates mussels from location C10 according to the relative levels of Fe and shell length.

Digestive gland

Axis 1 discriminates mussels from location C12 (Fig. 4A) according to the relative higher levels of Cd, Cu, Zn, and MT (Fig. 4B). Axis 2 discriminates positively Fe levels and negatively the shell length (Fig. 4B).

To summarize:

(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

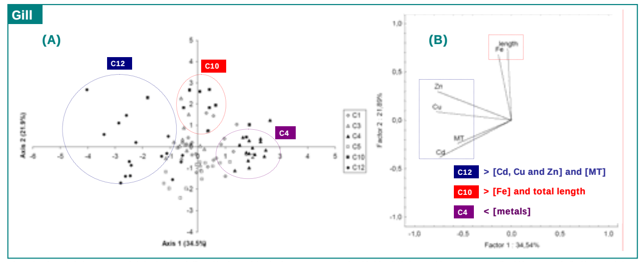


Fig. 3- First plane principal component analysis (PCA) of mussel gill metal levels at each location (C1, C3, C4, C5, C10 and C12)). (A) Individual scores. (B) Descriptor scores. All variables were standardized before analysis

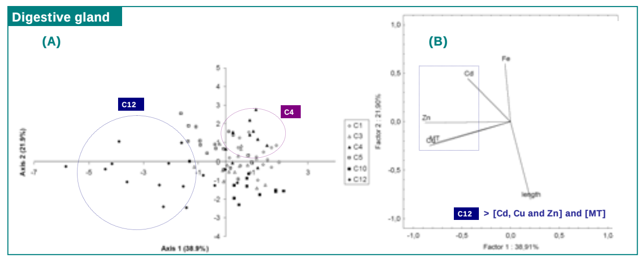


Fig. 4- First plane principal component analysis (PCA) of mussel digestive gland metal levels at each location (C1, C3, C4, C5, C10 and C12)). (A) Individual scores. (B) Descriptor scores. All variables were standardized before analysis.

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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