[OP 3.4]

Metal Compartmentalisation and Metallothioneins in the Shrimp, *Rimicaris exoculata*, from the Mid Atlantic Ridge; Preliminary Approach to the Fluid-Organism Relationship

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Goal and scope definition. The hydrothermal shrimps (Rimicaris exoculata, Alvinocarididae) have been observed in high density on active chimney walls at several sites (Snake Pit, 23°N, TAG, 26°N, Broken Spur, 29°N, Lucky Strike, 37°) on the Mid-Atlantic Ridge. They face an environment enriched with metals originating from the hydrothermal fluid.
Focuses / highlights. In order to analyse the fluid-organism relationship we investigated the relative abundance of metals in the water surrounding the shrimps and their accumulation by the shrimps.
Results. Samples were collected during the exploration of the Rainbow site (Marvel cruise, 1997) by the French submersible Nautile. Water samples were obtained using a multi sampler gathering four evacuated titanium 200 ml bottles manipulated by the submersible. Metal concentrations in water (Cu, Pb, Cd ; total dissolvable metal) were analysed by potentiometric stripping analysis. Metals in various anatomic parts (gills, digestive gland, abdomen) of *R. exoculata* were determined by flame (Cu, Fe, Mn, Zn) or electrothermal (Cd) atomic absorption spectrophotometry using the Zeeman effect. The pattern of their bioaccumulation in various anatomic parts of *R. exoculata* was compared to that of vent crustaceans from fast spreading ridge (East Pacific Rise, EPR) and coastal species to establish the hypothetical occurrence of a specific physiological adaptation to a metal-enriched environment. Tissues were homogenised and centrifuged in order to separate metals associated with insoluble compounds from those associated with soluble ones. Heat-denaturable compounds were separated from heat-stable compounds (including metallothioneins, MTs) by centrifugation of heated supernatants. Quantitative analyses of MTs were performed by differential pulse polarography in heat-denatured soluble fractions to assess their relative involvement in metal regulation. The amounts of total and heat-stable proteins were quantified respectively in the total supernatants and in the heat-denatured ones using the Lowry method.
This first approach of the metallic environment of *R. exoculata* showed a metallic enrichment and a great variability. This variability can be linked to the potentially high particulate contribution in the sample and the possible sampling artefact.
The levels of cadmium (toxic metal) within the anatomic parts were relatively low in comparison with those of the essential metals (copper, zinc) owing to cadmium lesser abundance within *R. exoculata* surrounding sea water.
Among the essential metals, manganese presented the lowest levels in the shrimp tissues owing to a lesser abundance or bioavailability in the surrounding water. The levels of zinc and iron were relatively high in comparison with those of copper.
Zinc levels in the gills and the digestive gland of *R. exoculata* were higher that those observed earlier for the same organs within the hydrothermal crab *Bythograea thermydron* from the East Pacific Rise. At the opposite the levels of copper in the digestive gland were lower. The comparison with the levels observed for the coastal crab (*Callinectes sapidus*) emphasised the bioaccumulation of metals in the tissues of the hydrothermal species but did not give any clue about its significance.
Likewise a comparison was established with metal levels in whole body of decapods collected in various locations. The levels of cadmium in the whole body of the decapods ranged in relation with their site of collection. The levels of cadmium in the gills and the digestive gland of Rimicaris were situated at the top of that range, while the abdomen level was at the bottom. With the exception of copper in the abdomen, the levels of copper and zinc observed for Rimicaris were higher than those recorded for non-hydrothermal decapods.
With the exception of iron and copper equally abundant under both forms within the digestive gland, and that of copper in the abdomen, metals were essentially under insoluble forms. But concerning the gills (Cd and Zn) and the abdomen (Fe, Cd and Zn) we could not reject the hypothesis of an external deposit of particulate material enriched with metals. In comparison with the data from B. thermydron, Rimicaris showed lower ratios of soluble metals within the gills and the digestive gland, with the exception of copper within the gills.
The level of MTs recorded for Rimicaris abdomen was low owing to the little involvement of that kind of organ (muscles) to metal metabolism in general. The ratios of MTs versus total or heat-stable proteins were higher in the digestive gland than in the gills, in relation with a higher ratio of soluble metals, taking in account that in the gills the existence of external deposits could result in a bias.

Conclusion. We did not evidence any unsuspected mechanism for metal storage and detoxification. Unless informations concerning the relative abundance of particule metals in the surrounding water we can assume that Rimicaris have developed efficient metal-detoxication processes under insoluble forms.
Outlook. However, this study pointed out the improvements needed by the sampling method that should differentiate between the dissolved and particulate phase with an in-situ filtration of the sample for the water surrounding vent organisms and for the preparation of the biological samples. It also emphasised the importance of developing on board experimentations to study metal uptake and metabolism.

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