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Development of an ecotoxicological protocol for the deep-sea fauna using the hydrothermal vent shrimp *Rimicaris exoculata*

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ABSTRACT

In light of deep-sea mining industry development, particularly interested in massive-sulphide deposits enriched in metals with high commercial value, efforts are increasing to better understand potential environmental impacts to local fauna. The aim of this study was to assess the natural background levels of biomarkers in the hydrothermal vent shrimp *Rimicaris exoculata* and their responses to copper exposure at *in situ* pressure (30 MPa) as well as the effects of depressurization and pressurization of the high-pressure aquarium IPOCAMP. *R. exoculata* were collected from the chimney walls of the hydrothermal vent site TAG (Mid Atlantic Ridge) at 3630 m depth during the BICOSE cruise in 2014. Tissue metal accumulation was quantified in different tissues (gills, hepatopancreas and muscle) and a battery of biomarkers was measured: metal exposure (metallothioneins), oxidative stress (catalase, superoxide dismutase, glutathione-*S*-transferase and glutathione peroxidase) and oxidative damage (lipid peroxidation). Data show a higher concentration of Cu in the hepatopancreas and a slight increase in the gills after incubations (for both exposed groups). Significant induction of metallothioneins was observed in the gills of shrimps exposed to 4 μM of Cu compared to the control group. Moreover, activities of enzymes were detected for the *in situ* group, showing a background protection against metal toxicity. Results suggest that the proposed method, including a physiologically critical step of pressurizing and depressurizing the test chamber to enable the seawater exchange during exposure to contaminants, is not affecting metal accumulation and biomarkers response and may prove a useful method to assess toxicity of contaminants in deep-sea species.

1. Introduction

Hydrothermal vents are massive sulphide deposits with high concentration of various metals (*e.g.* Cu, Ni, Zn, Mn, Co, Ag, Au) with potential economic interest to mining industries (Hannington et al., 2011). Recently, the Government of Papua New Guinea has given exploration license to Nautilus Minerals Corporation to explore this type of mineral deposits in the Manus basin (Gena, 2013). However, it is likely that the impact of human activities will somehow change the natural equilibrium of this particular environment (Van Dover, 2014). The living fauna will likely be exposed to variations in environmental metal composition, hence the need to further explore the toxicity of chemicals associated with deep-sea exploitation. For this it is considered essential to adapt the existing ecotoxicological protocols from shallow-water to the deep-sea conditions to enable a better understanding of potential anthropogenic impact on deep-sea fauna (Mestre et al., 2014).

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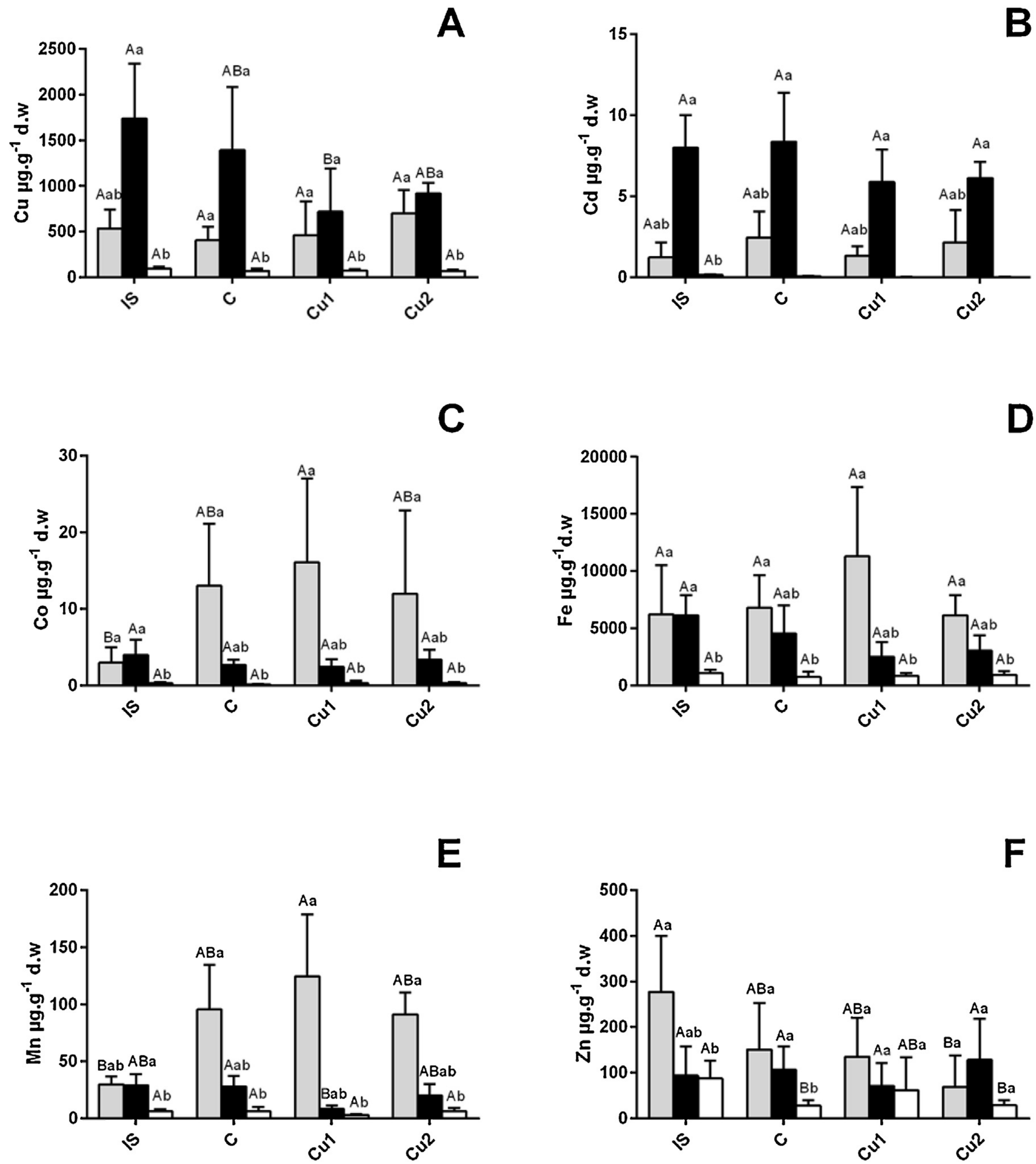

Fig. 1. Metals concentrations (mean ± SD) in tissues of *Rimicaris exoculata* for the different treatments. Values followed by the same letter are not significantly different ($p > 0.05$) with capital letters for comparison of one tissue between groups and lowercase letters for comparison among one group. Gills (grey), hepatopancreas (black), abdominal muscle (white), IS: *in situ* group, C: control, Cu1: exposed to 0.4 µM, Cu2: exposed to 4 µM.

The Trans-Atlantic Geotraverse (TAG) hydrothermal vent field is located on the Mid-Atlantic Ridge and its hot fluids are rich in various metals, including copper with concentrations ranging between 120 and 150 µM (Edmonds et al., 1996). Copper is an essential trace element found in a variety of cells and tissues and used as cofactor of several enzymes. However, free Cu ions (Cu^+ and Cu^{2+}) can participate in the formation of reactive oxygen species (ROS) (Gaetke and Chow, 2003). As invertebrates possess the ability to concentrate certain metals from the surrounding medium, reflecting the variation in metal concentration in the environment (White and Rainbow, 1985), they are useful tools to monitor change of metal amounts as a result of anthropogenic activities.

Increasing total dissolved metal concentration will lead to a higher metal uptake rate (Rainbow, 1998). To prevent the increase of metal accumulation in cells, organisms possess defence mechanisms such as specific proteins which play a role in binding metals, as for example the metallothioneins (MTs). These cysteine-rich non-enzymatic proteins are vital for various biological functions like storage, transport, compartmentalization of essential and non-essential metals (detoxification mechanisms) and can also act as oxyradicals scavengers protecting cells against ROS (Roesijadi, 1992). In the presence of certain metals and/or high-metals concentrations, ROS formation will induce an antioxidant defence mechanism. This mechanism consists in the action of specific enzymatic proteins, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-*S*-transferase (GST). These enzymes will limit the ROS levels and prevent oxidative damage (Di Giulio et al., 1995). Nevertheless, if the action of these