



**HAL**  
open science

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

M. Auguste, N.C. Mestre, T.L. Rocha, C. Cardoso, V. Cueff-Gauchard, S. Le Bloa, M.A. Cambon-Bonavita, B. Shillito, M. Zbinden, J. Ravaux, et al.

### ► To cite this version:

M. Auguste, N.C. Mestre, T.L. Rocha, C. Cardoso, V. Cueff-Gauchard, et al.. Development of an ecotoxicological protocol for the deep-sea fauna using the hydrothermal vent shrimp *Rimicaris exoculata*. *Aquatic Toxicology*, 2016, 175, pp.277-285. 10.1016/j.aquatox.2016.03.024 . hal-03800831

**HAL Id: hal-03800831**

**<https://hal.science/hal-03800831>**

Submitted on 6 Oct 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

3 M. Auguste<sup>a</sup>, N.C. Mestre<sup>a\*</sup>, T.L Rocha<sup>a</sup>, C. Cardoso<sup>a</sup>, V. Cueff-Gauchard<sup>b</sup>, S. Le Bloa<sup>b</sup>,  
4 M.A. Cambon-Bonavita<sup>b</sup>, B. Shillito<sup>c</sup>, M. Zbinden<sup>c</sup>, J. Ravaux<sup>c</sup>, M. J. Bebianno<sup>a</sup>

6 <sup>a</sup>CIMA, Faculty of Science and Technology, University of Algarve, Campus de  
7 Gambelas, 8005 139 Faro, Portugal.

8 <sup>b</sup>Ifremer, REM/EEP/Laboratoire de Microbiologie des Environnements Extrêmes, UMR  
9 6197, Ifremer, UBO, CNRS, Technopôle Brest Iroise, CS10070, Plouzané, France

10 <sup>c</sup>Sorbonne Universités, UPMC Univ Paris 06, UMR CNRS 7208, MNHN/IRD-  
11 207/UCN/UA, Biologie des Organismes et Ecosystèmes Aquatiques", 7 Quai St  
12 Bernard, 75252 Cedex 5 Paris, France.

13 \*Corresponding author at: N.C. Mestre, CIMA, Faculty of Science and Technology,  
14 University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal. Tel.: +351  
15 289800100; Fax: + 351 289800069. *E-mail address*: neliamestre@gmail.com

18 **ABSTRACT**

19           In light of deep-sea mining industry development, particularly interested in  
20 massive-sulphide deposits enriched in metals with high commercial value, efforts are  
21 increasing to better understand potential environmental impacts to local fauna. The aim  
22 of this study was to assess the natural background levels of biomarkers in the  
23 hydrothermal vent shrimp *Rimicaris exoculata* and their responses to copper exposure at  
24 *in situ* pressure (30 MPa) as well as the effects of depressurization and pressurization of  
25 the high-pressure aquarium IPOCAMP. *R. exoculata* were collected from the chimney  
26 walls of the hydrothermal vent site TAG (Mid Atlantic Ridge) at 3630 m depth during  
27 the BICOSE cruise in 2014. Tissue metal accumulation was quantified in different  
28 tissues (gills, hepatopancreas and muscle) and a battery of biomarkers was measured:  
29 metal exposure (metallothioneins), oxidative stress (catalase, superoxide dismutase,  
30 glutathione-S-transferase and glutathione peroxidase) and oxidative damage (lipid  
31 peroxidation). Data show a higher concentration of Cu in the hepatopancreas and a

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32 slight increase in the gills after incubations (for both exposed groups). Significant  
33 induction of metallothioneins was observed in the gills of shrimps exposed to 4  $\mu\text{M}$  of  
34 Cu compared to the control group. Moreover, activities of enzymes were detected for  
35 the *in situ* group, showing a background protection against metal toxicity. Results  
36 suggest that the proposed method, including a physiologically critical step of  
37 pressurizing and depressurizing the test chamber to enable the seawater exchange  
38 during exposure to contaminants, is not affecting metal accumulation and biomarkers  
39 response and may prove a useful method to assess toxicity of contaminants in deep-sea  
40 species.

41  
42 *Keywords:* Deep-sea mining; hydrothermal vents; biomarkers; IPOCAMP; copper;  
43 TAG.

## 44 45 46 **1. Introduction**

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

59 The Trans-Atlantic Geotraverse (TAG) hydrothermal vent field is located on the  
60 Mid-Atlantic Ridge and its hot fluids are rich in various metals, including copper with  
61 concentrations ranging between 120 and 150  $\mu\text{M}$  (Edmonds et al. 1996). Copper is an  
62 essential trace element found in a variety of cells and tissues and used as cofactor of  
63 several enzymes. However, free Cu ions ( $\text{Cu}^+$  and  $\text{Cu}^{2+}$ ) can participate in the formation

64 of reactive oxygen species (ROS) (Gaetke and Chow 2003). As invertebrates possess  
65 the ability to concentrate certain metals from the surrounding medium, reflecting the  
66 variation in metal concentration in the environment (White and Rainbow 1985), they are  
67 useful tools to monitor change of metal amounts as a result of anthropogenic activities.

68         Increasing total dissolved metal concentration will lead to a higher metal uptake  
69 rate (Rainbow 1998). To prevent the increase of metal accumulation in cells, organisms  
70 possess defence mechanisms such as specific proteins which play a role in binding  
71 metals, as for example the metallothioneins (MTs). These cysteine-rich non-enzymatic  
72 proteins are vital for various biological functions like storage, transport,  
73 compartmentalization of essential and non-essential metals (detoxification mechanisms)  
74 and can also act as oxyradicals scavengers protecting cells against ROS (Roesijadi  
75 1992). In the presence of certain metals and/or high-metals concentrations, ROS  
76 formation will induce an antioxidant defence mechanism. This mechanism consists in  
77 the action of specific enzymatic proteins, such as superoxide dismutase (SOD), catalase  
78 (CAT), glutathione peroxidase (GPx) and glutathione-s-transferase (GST). These  
79 enzymes will limit the ROS levels and prevent oxidative damage (Di Giulio et al. 1995).  
80 Nevertheless, if the action of these enzymes is not sufficient, the free radicals will reach  
81 the cellular membranes and cause oxidative damage, such as lipid peroxidation (LPO),  
82 which is the oxidation of polyunsaturated fatty acids (PUFAs; Halliwell and Gutteridge  
83 1984).

84         Previous studies have already looked into metal accumulation and antioxidant  
85 responses in the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus* from  
86 Menez-Gwen, Lucky Strike and Rainbow vent sites (Company et al. 2004, 2006a,b,  
87 2007, 2008, Bebianno et al. 2005). A few studies have looked into metal accumulation  
88 (Kádár et al. 2006, Geret et al. 2002), antioxidant enzymes activities (Gonzalez-Rey et  
89 al. 2008), MTs and oxidative damage (Geret et al. 2002, Gonzalez-Rey et al. 2007) in  
90 the vent shrimp *Rimicaris exoculata*. Nevertheless, only one study has investigated the  
91 toxicity of metal exposure under laboratory controlled deep-sea conditions of high  
92 pressure and low temperature (Company et al. 2006a) using one of the few available  
93 equipment that enable these kind of studies, the IPOCAMP (Incubateur Pressurisé pour  
94 l'Observation et la Culture d'Animaux Marins Profonds; Shillito et al. 2014).

95         The chimney walls at TAG are covered by dense swarms (1200-3000 indiv.m<sup>2</sup>)  
96 of the vent shrimp *R. exoculata*, also known to be one of the most abundant invertebrate

1 97 species in the Mid-Atlantic Ridge (Desbruyères et al. 2000, Schmidt et al. 2008). *R.*  
2 98 *exoculata* is also one of the most studied deep-sea species, making it a good model for  
3 99 investigating deep-sea environmental changes (e.g. Van Dover et al. 1988, Gebruk et al.  
4 100 1993, Vereshchaka 1997, Rieley et al. 1999, Allen et al. 2001, Geret et al. 2002, Ravaux  
5 101 et al. 2003, Zbinden et al. 2004, 2008, Copley et al. 2007, Gonzalez-Rey et al. 2007,  
6 102 2008, Anderson et al. 2008, Corbari et al. 2008, Schmidt et al. 2008, 2009, Cottin et al.  
7 103 2010, Ponsard et al. 2013, Jan et al. 2015).

8 104 Elutriate toxicity tests with weathered vent chimney material were conducted  
9 105 during the environmental impact studies at Solwara 1, Papua New Guinea. The  
10 106 concentration of Cu released after 30 min in the field-based elutriate tests was 15-20  
11 107  $\mu\text{g.L}^{-1}$  and in the laboratory-based tests was 38-140  $\mu\text{g.L}^{-1}$  (Simpson et al. 2008). The  
12 108 selected copper concentrations used in the present study reflect these results.

13 109 This study assesses the biomarkers (MTs, antioxidant enzymes and LPO)  
14 110 response in different tissues (gills, hepatopancreas and abdominal muscle) in *R.*  
15 111 *exoculata* exposed to 0.4  $\mu\text{M}$  (25  $\mu\text{g.L}^{-1}$ ) and 4  $\mu\text{M}$  (254  $\mu\text{g.L}^{-1}$ ) of copper at *in situ*  
16 112 pressure and temperature (30 MPa, 10°C). It also compares the metal accumulation (Cu,  
17 113 Zn, Fe, Mn, Co, Cd) and biomarkers baseline levels under natural conditions (isobaric  
18 114 sampling) and after maintenance in a high-pressure aquarium IPOCAMP (no metal  
19 115 exposure). Finally, this study also compares the effects of depressurization and  
20 116 pressurization of the test chamber (conditions necessary for the Cu enriched seawater  
21 117 and oxygen renewal during the test).

22 118

23 119

## 24 120 **2. Materials and Methods**

### 25 121 **2.1. Sample collection**

26 122 *Rimicaris exoculata* were sampled in February 2014 from the TAG vent field  
27 123 (26° 08.2' N, 44° 49.5' W) in the Mid-Atlantic Ridge, during the oceanographic cruise  
28 124 BICOSE, aboard the research vessel *Pourquoi pas?*. The organisms were collected  
29 125 using the slurp gun of the ROV VICTOR 6000 (IFREMER), and using the PERISCOP  
30 126 for isobaric and isothermal recovery (Shillito et al. 2008). The shrimps were taken from  
31 127 the main black smoker on the wall near the main spire.

128

## 129 **2.2. Cu exposure experiments**

130 A total of 24 shrimps were used in this study, divided into 4 groups. The first  
131 group (n=6) called *in situ* (IS) was dissected under sterile conditions and flash frozen in  
132 liquid nitrogen straight after collection and stored at -80°C until further use. The other  
133 shrimps were directly transferred into the pressurized aquarium IPOCAMP for a Cu  
134 exposure experiment at 10°C. A copper solution was prepared using CuSO<sub>4</sub> (analytical  
135 grade, Sigma). Shrimps (n= 6 *per* condition) were isolated in 3.8 L boxes for: control  
136 (C; only seawater); 0.4 µM of Cu (Cu1); and 4 µM of Cu (Cu2). The experiment was  
137 carried out at 30 MPa and 10°C for 72 h. For seawater and oxygen renewal, the  
138 IPOCAMP was depressurized every 12 h, and the viability of the animals was checked.  
139 At the end of the exposure period (72 h), survival of the shrimp was 100%, although  
140 one of the animals of the Cu2 group appeared less responsive to stimulation. The tissues  
141 were dissected (gills, hepatopancreas and muscle; without gut or exoskeleton) and  
142 immediately frozen in liquid nitrogen and stored at -80°C until further analyses.

## 144 **2.3. Tissue preparation**

145 Tissues were individually homogenized at 4°C in a Tris-HCl (0.02 M, 5 ml.g<sup>-1</sup>  
146 soft tissue) buffer with butylated hydroxytoluene (BHT, 10 µl.mL<sup>-1</sup>), pH 8.6. The  
147 homogenate (3 ml) was separated in soluble and insoluble fractions by centrifugation  
148 (30000×g, 30 min, 4°C) and the remaining homogenate was used for determination of  
149 metal concentrations. After centrifugation, a part of the supernatant was used for the  
150 measurement of LPO and total protein content. A second centrifugation (30000×g, 30  
151 min, 4°C) separated the low molecular weight proteins, and the supernatant was used  
152 for MT measurements.

153 For antioxidant enzyme analysis, tissues were individually homogenized in 50  
154 mM Tris-HCl buffer, pH 7.6, containing sucrose (250 mM), MgCl<sub>2</sub> (5 mM), DTT (1  
155 mM). After 10 min of incubation, the homogenates were centrifuged at 1000×g for 10  
156 min at 4°C and the cytosolic fraction was kept for analysis.

## 158 **2.4. Metal analysis**

159 For metal concentration determination, homogenates were weighed, dried (80°C,  
160 48 h) and submitted to wet acid digestion with 67% nitric acid in a hot plate (80°C, 2 h).  
161 Six metals (Cu, Zn, Fe, Mn, Co, Cd) were analysed by graphite furnace absorption  
162 spectrometry (AAS, AAnalyst 800- PerkinElmer). Accuracy of the analytical method  
163 was checked by analysing certified reference material TORT-2 (NRC-CNRC) (lobster  
164 hepatopancreas), measured values ( $99 \pm 17 \mu\text{g.g}^{-1}$  Cu ,  $138 \pm 28$  Fe,  $124 \pm 52$  Zn,  $14 \pm 2$   
165 Mn,  $0.49 \pm 0.08$  Co,  $33 \pm 3$  Cd) were within the values of certified values in the  
166 reference material ( $106 \pm 10 \mu\text{g.g}^{-1}$  Cu,  $105 \pm 13$  Fe,  $180 \pm 6$  Zn,  $13.6 \pm 1.2$  Mn,  
167  $0.51 \pm 0.09$  Co,  $26.7 \pm 0.6$  Cd). Data expressed as  $\mu\text{g.g}^{-1}$  of dry tissue weight (d.w.). The  
168 values for total soft tissues correspond to the sum of gills, hepatopancreas and muscle,  
169 taking into the account the contribution of the weight of each tissue.

170

## 171 **2.5. Metallothioneins (MTs)**

172 MTs were determined in the heat-stable supernatant by differential normal pulse  
173 polarography (Bebianno and Langston 1989), using an Eco Chemie mAutolab III  
174 potentiostat and a Methrom 663VA stand with a static mercury drop electrode (SMDE)  
175 and GPES 4.9 software (Eco Chemie). In the absence of a shrimp MT standard, a rabbit  
176 MT standard was used. MTs levels are expressed in  $\mu\text{g g}^{-1}$  of wet tissue weight (w.w.).

177

## 178 **2.6. Total proteins**

179 Total proteins were measured by the method of Bradford et al. (1976) adapted to  
180 a microplate reader (Infinite® 200 PRO-Tecan), using Bovine Serum Albumin (Sigma-  
181 Aldrich) as a standard. The proteins concentration is expressed as  $\text{mg.g}^{-1}$  of wet tissue  
182 weight.

183

## 184 **2.7. Antioxidant enzymes activities**

185 Superoxide dismutase (SOD) activity was determined by measuring the  
186 reduction of cytochrome c by the xanthine oxidase/hypoxanthine system at 550 nm  
187 (McCord and Fridovich 1969). One unit of SOD is defined as the amount of enzyme  
188 that inhibits the reduction of cytochrome c by 50%. SOD activity is expressed in  $\text{U.mg}^{-1}$   
189 prot.

190 Catalase (CAT) activity was determined according to Greenwald (1985). The  
191 activity was determined as the decrease in absorbance during 1 min at 240 nm due to  
192 H<sub>2</sub>O<sub>2</sub> consumption in buffer of monobasic and dibasic potassium, pH 7.5. CAT activity  
193 is expressed as  $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$  prot.

194 Glutathione peroxidation (GPx) activity was measured by following NADPH  
195 oxidation at 340 nm in the presence of excess glutathione reductase, reduced glutathione  
196 and corresponding peroxide (Flohe and Gunzler 1984). The GPx activities were  
197 measured by using cumene hydroperoxide as substrate. GPx activity is expressed as  
198  $\text{nmol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$  prot.

199 Glutathione-S-transferase (GST) activity was determined by following the  
200 conjugation of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB;  
201 Habig et al. 1974). A buffer containing KH<sub>2</sub>PO<sub>4</sub>/ K<sub>2</sub>HPO<sub>4</sub> (0.2 M), pH 7.9, was used.  
202 The change in absorbance was recorded at 340 nm for 1 min. The activity was  
203 calculated as nmol of CDNB formed per  $\text{min}^{-1}\cdot\text{mg}^{-1}$  prot (using a molar extinction  
204 coefficient of  $9.6\text{ mM}^{-1}\cdot\text{cm}^{-1}$ ).

## 206 **2.8. Lipid peroxidation (LPO)**

207 Oxidative damage was analysed in terms of LPO. Determination of  
208 malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE) concentration, both sub-  
209 products of polyunsaturated fatty acid peroxidation, was realized using the method  
210 described by Erdelmeier et al. (1998) and maximal absorbance at 586 nm.  
211 Malondialdehyde bis-(dimethyl acetal) (Sigma-Aldrich) was used as a standard. The  
212 LPO is expressed in nmol of MDA + 4-HNE  $\text{mg}^{-1}$  prot.

## 214 **2.9. Statistical analysis**

215 The non-parametric Kruskal-Wallis test was used to assess the significant  
216 differences between exposure groups and tissues for each variable (biomarkers and  
217 metals concentration). Results were significantly different when  $p<0.05$ . Principal  
218 components analysis (PCA) was used in the ordination method to evaluate the  
219 relationship between the different treatments and the analysed variables in the gills and  
220 the hepatopancreas.



221

222

### 223 3. Result and Discussion

#### 224 3.1. Metal accumulation

225 The baseline metals concentrations in *R. exoculata* collected from TAG for the  
226 different tissues are described in the Table 1. In all tissues the metal concentration  
227 descending trend is the following Fe > Cu > Zn > Mn. In the total soft tissues, gills and  
228 muscle Co is more abundant than Cd, and the opposite was noted in the hepatopancreas  
229 (Table 1). The TAG black smoker presents hot fluids rich in metals (Fe 5590  $\mu\text{M}$ , Cu  
230 120-150  $\mu\text{M}$ , Zn 46  $\mu\text{M}$ , Mn 680  $\mu\text{M}$ , Co <2  $\mu\text{M}$ , Cd 66 nM) what is reflected by the  
231 accumulation of these metals in *R. exoculata* tissues (Table 1). This is in agreement  
232 with previous studies, with the same species but from another Mid-Atlantic Ridge vent  
233 field - Rainbow (hot fluids metal contents: Fe 24000  $\mu\text{M}$ , Cu 140  $\mu\text{M}$ , Zn 160  $\mu\text{M}$ , Mn  
234 2250  $\mu\text{M}$ , Co 13  $\mu\text{M}$ , Cd 130 nM; Douville et al. 2002). At both sites, the concentration  
235 of metals in different tissues is coherent with the variation of metals in the hot fluids.  
236 Rainbow's hot fluid is much richer in iron than TAG's fluid and iron accumulation is  
237 higher in shrimp's gills from the Rainbow vent site (90460  $\mu\text{g}\cdot\text{g}^{-1}$  d.w.; Kádár et al.  
238 2006) when compared to those from TAG (6201  $\mu\text{g}\cdot\text{g}^{-1}$  d.w.; Table 1).

239 Vent shrimps do show higher natural Cu concentration in their body (379  $\mu\text{g}\cdot\text{g}^{-1}$   
240 d.w.) than their coastal counterparts ( $\sim 100\mu\text{g}\cdot\text{g}^{-1}$  d.w.; White and Rainbow 1985). In a  
241 review by Pourang et al. (2004), the distribution of trace elements on coastal shrimps  
242 were listed, and for Cu, gills always show higher Cu concentrations, followed by  
243 hepatopancreas and then muscle. Nevertheless, in the present study, the hepatopancreas  
244 showed higher Cu concentration, and this observation is different from other studies  
245 where gills presented the highest concentration for the same species (Kádár et al. 2006,  
246 Geret et al. 2002). However, the present results are consistent with other studies on vent  
247 crustaceans, such as the accumulation of trace metals in the crab *Bythograea*  
248 *thermydrion* where the highest Cu levels were found in the hepatopancreas (Cosson and  
249 Vivier 1997). The high amount of Cu present in the hepatopancreas can be explained by  
250 the presence of high amounts of haemocyanin, copper-sulphur containing granules and  
251 MTs. Haemocyanin is one of the main protein synthesized in this organ (could represent  
252 50% of total protein synthesized; Viarengo and Nott 1993).

253 For the copper exposure experiment, Cu concentrations in the tissues for the  
254 different treatments are shown in the Figure 1 (see also Table A.1 in supplementary  
255 material). The results do not show significant differences between groups ( $p>0.05$ ),  
256 except for the zinc with higher concentrations in the gills ( $277 \mu\text{g.g}^{-1}$  d.w.) and in the  
257 total soft tissues ( $112 \mu\text{g.g}^{-1}$  d.w.) of the *in situ* group when compared to shrimps  
258 exposed to  $4 \mu\text{M}$  of Cu ( $69 \mu\text{g.g}^{-1}$  d.w. and  $49 \mu\text{g.g}^{-1}$  d.w., respectively;  $p<0.05$ ). For the  
259 *in situ* group, the concentration of Co and Mn is similar in the hepatopancreas and gills,  
260 but in the groups incubated inside the IPOCAMP there is a higher concentration in the  
261 gills. Cd is mainly accumulated in the hepatopancreas ( $8.3 \mu\text{g.g}^{-1}$  d.w.), when compared  
262 to the gills ( $1.2 \mu\text{g.g}^{-1}$  d.w) and the muscle ( $0.15 \mu\text{g.g}^{-1}$  d.w.; Fig. 1). As the *in situ*  
263 group represents the baseline concentrations, these results suggest that after incubation  
264 inside the IPOCAMP, where only Cu was added but not the other metals, there is a  
265 redistribution of metals among the different tissues. As some of these are essential  
266 metals, these results suggest their potential use in other biological functions in the gills,  
267 or that their excess are further eliminated/regulated through the gills.

268 A high variability in Cu concentration among the shrimp replicates was noted  
269 (Fig. 1). Still, the Cu concentration in the hepatopancreas decreases in the groups  
270 exposed to Cu (Cu1:  $440 \mu\text{g.g}^{-1}$  d.w., Cu2:  $810 \mu\text{g.g}^{-1}$  d.w.) when compared to the *in*  
271 *situ* and control groups (IS:  $1500 \mu\text{g.g}^{-1}$  d.w., C:  $1376 \mu\text{g.g}^{-1}$  d.w.). On the other hand, a  
272 slight increase in Cu concentration is observed in the gills for the Cu exposed groups  
273 while a non-significant decrease in Cu present in the muscle is noticed ( $p>0.05$ ; Fig. 1).  
274 Although, there is no published copper concentration values for *R. exoculata* habitat at  
275 TAG, the present results observed for the hepatopancreas suggest an elimination  
276 process during the 72 h incubation. Crustaceans possess Cu sulphur granules in the  
277 hepatopancreas and these may play a role as a detoxification mechanism. In shrimps,  
278 copper and sulphur are accumulated in the epithelial cells of the hepatopancreas as  
279 insoluble granules, thus decreasing the potential toxic effect of Cu present in the  
280 cytoplasm of cells (Viarengo and Nott 1993, Ahearn et al. 2004). Moreover, the total Cu  
281 amount for the two exposed groups do not show significant differences which is in  
282 favour of a potential regulation mechanism of metal metabolism by the shrimps. White  
283 and Rainbow (1982) found that *Palaemon elegans* are able to regulate body levels of  
284 copper up to a threshold exposure level, beyond which the metal is accumulated in  
285 direct proportion to external metal levels (up to  $100 \mu\text{g.L}^{-1}$  for the coastal shrimp). This

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

286 threshold would presumably be higher for vent shrimps as they are constantly bathed in  
287 seawater rich in metals. This regulation can result from a balance between accumulation  
288 and elimination rate.

289

### 290 **3.2. Metallothioneins**

291 The MTs levels increased in the gills of the Cu exposed groups when compared  
292 to control and IS, with significant differences between the control ( $155 \mu\text{g.g}^{-1}$  w.w.) and  
293 Cu2 ( $332 \mu\text{g.g}^{-1}$  w.w.;  $p < 0.05$ ; Fig. 2). Higher amounts of MTs ( $400 \mu\text{g.g}^{-1}$  w.w.) were  
294 observed in the hepatopancreas (Fig. 2) while the muscle had the lowest, but in both  
295 tissues no significant differences were observed between the different treatments  
296 ( $p > 0.05$ ).

297 To our knowledge only two studies on MTs levels in *R. exoculata* were  
298 previously reported. The study by Geret et al. (2002) presents data of MTs per tissues  
299 for *R. exoculata* from the Rainbow hydrothermal vent field. The MT data obtained in  
300 the present study (gills:  $161 \mu\text{g.g}^{-1}$  w.w.; muscle:  $27 \mu\text{g.g}^{-1}$  w.w.; Fig. 2) are in the same  
301 order of magnitude as for *R. exoculata* from the Rainbow (gills:  $255 \mu\text{g.g}^{-1}$  w.w.;  
302 muscle:  $72 \mu\text{g.g}^{-1}$  w.w.; Geret et al. 2002). However, TAG shrimps have lower MT  
303 levels in the hepatopancreas ( $399 \mu\text{g.g}^{-1}$  w.w.) than those from Rainbow ( $3012 \mu\text{g.g}^{-1}$   
304 w.w.; Geret et al. 2002). The other study that investigated MTs levels analysed the soft  
305 tissues of *R. exoculata* and *Mirocaris fortunata* which do not allow a direct comparison  
306 with the present, per tissue, results (Gonzalez-Rey et al. 2008).

307 MTs are induced in response to the Cu exposure. The significant induction of  
308 MTs in the gills at higher Cu concentration ( $4 \mu\text{M}$ ) when compared to the control group  
309 seems to follow the slight increase of Cu (Figs. 1 and 2). This is probably linked the role  
310 of MTs in the detoxification and/or storage of Cu. An antioxidant role for MTs was  
311 suggested in previous studies with the mussel *Mytilus galloprovincialis*, through  
312 oxyradical scavenging protecting both isolated cells and the entire organism from  
313 oxidative stress (Viarengo et al. 1999). In the hepatopancreas, MTs levels do not change  
314 between groups ( $p > 0.05$ ). Different explanations may be proposed. First, Campana et al.  
315 (2015) suggest that in the shrimp *Pandalus montagui*, most of the Cu is accumulated in  
316 a metabolically available form and excretion occurs from this component, implying that  
317 Cu could be eliminated in a metabolically active form and so would not induce MTs

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

318 response. On the other hand, MTs can be linked to Cd, which is mainly accumulated in  
319 the hepatopancreas (Fig. 1) and whose amount does not vary after 72h of incubation.  
320 The affinity of MTs with metals defines their binding abilities (Cu>Cd>Zn; Engel and  
321 Brouwer 1989). Cd is a non-essential metal, thus to avoid toxicity the organisms have to  
322 limit their accumulation or to complex the accumulated metal to a non- or less-toxic  
323 form (Depledge and Rainbow 1990).

324

### 325 **3.3. Antioxidant enzymatic response**

326 The antioxidant enzymes activities show a tissue specific response and the  
327 separate analysis per tissue (not whole organism) is recommended for future studies, for  
328 higher resolution of the results. SOD activity is higher in the hepatopancreas with IS  
329 and C groups showing the highest values (IS: 95 U.mg<sup>-1</sup> prot, C: 123 U.mg<sup>-1</sup> prot; Fig.  
330 3) and following the trend of Cu concentration (Table 1 and Fig. 1). This is in line with  
331 previous suggestions that the increase in cytosolic SOD activity is stimulated by higher  
332 Cu concentrations (Greco et al. 1990, Li et al. 2006). In the gills, no significant change  
333 was noted between the different treatments ( $p>0.05$ ). The highest GPx activity is  
334 observed in the hepatopancreas of the *in situ* group (706 nmol.min<sup>-1</sup>.mg<sup>-1</sup> prot; Fig. 3),  
335 which is significantly higher than in control (366 nmol.min<sup>-1</sup>.mg<sup>-1</sup> prot,  $p<0.05$ ).

336 Both CAT and GST activities follow a similar pattern (Fig. 3). An increasing  
337 trend of these enzymes activities in the gills follow the increasing Cu concentration (IS:  
338 1.3 μmol CAT.min<sup>-1</sup>.mg<sup>-1</sup> prot, 116 μmol GST.min<sup>-1</sup>.mg<sup>-1</sup> prot, Cu2: 2.8 μmol  
339 CAT.min<sup>-1</sup>.mg<sup>-1</sup> prot, 301 μmol GST.min<sup>-1</sup>.mg<sup>-1</sup> prot) but are not significantly different  
340 ( $p>0.05$ ). Increasing CAT activity with increasing Cu concentration has been previously  
341 observed (Li et al. 2006). Due to the low amount of tissue available, it was not possible  
342 to determine the CAT activity for the hepatopancreas. The GST activity in the  
343 hepatopancreas for the *in situ* group (225 μmol.min<sup>-1</sup>.mg<sup>-1</sup> prot) was significantly lower  
344 when compared to the control group (406 μmol.min<sup>-1</sup>.mg<sup>-1</sup> prot,  $p<0.05$ ). Moreover, for  
345 the groups placed in the IPOCAMP, a slight downward trend in GST activity was  
346 observed with increasing Cu concentration. CAT and GST activities in the muscle were  
347 higher for the *in situ* and Cu1 groups, with Cu1 significantly higher than Cu2 for both  
348 enzymes ( $p<0.05$ ; Fig. 3). These enzyme activities detected in the tissues of shrimps  
349 suggest a protective mechanism against the presence of ROS, as previously noted by

350 Gonzalez-Rey et al. (2008). In their study, they found that CAT in soft tissues of  
351 shallow-water shrimps as lower activity than in vents shrimps, while higher values of  
352 SOD and GPx activities were found in shallow-water shrimps (Gonzalez-Rey et al.  
353 2008; Table 2). When comparing the enzyme activities in the hepatopancreas, shallow-  
354 water shrimps show lower activity for GPx (8.8 and 4.71 nmol.min.mg<sup>-1</sup>.prot for *P.*  
355 *serratus*; Oliveira et al. 2012, Silva et al. 2013) and GST (6.4 and 3.1 nmol.min.mg<sup>-1</sup>  
356 <sup>1</sup>.prot for *P. serratus*; Oliveira et al. 2012, Silva et al. 2013) when compared to *R.*  
357 *exoculata* (GPx: 804.2 nmol.min.mg<sup>-1</sup>.prot, GST: 225.6 nmol.min.mg<sup>-1</sup>.prot; Ttable 2).  
358 Similarly, *R. exoculata* presents higher values of CAT activity in the muscle (0.55  
359 μmol.min.mg<sup>-1</sup>.prot) when compared to *P. elegans* (0.0053 μmol.min.mg<sup>-1</sup>.prot for;  
360 Vinagre et al. 2014; Table 2). These observations are suggesting a detoxification  
361 strategy in this vent shrimp, adapted to an enriched metal extreme environment.

362

### 363 **3.4. Oxidative damage**

364 The LPO level in the gills of the *in situ* shrimps (1.3 nmol.mg<sup>-1</sup> prot) was lower  
365 than in the other groups (Fig. 4). The control group shows higher damage (2.3 nmol.mg<sup>-1</sup>  
366 <sup>1</sup> protein) in the gills followed by a smooth decreasing trend of LPO levels with  
367 increasing Cu concentration (Cu1: 2 nmol.mg<sup>-1</sup> prot, Cu2: 1.7 nmol.mg<sup>-1</sup> prot), although  
368 no significant difference was found ( $p>0.05$ ). LPO in the muscle is similar for the two  
369 groups exposed to Cu (0.50 and 0.54 nmol.mg<sup>-1</sup> prot for Cu1 and Cu2 respectively;  
370  $p>0.05$ ) while in the *in situ* group it is more than 1.5-fold higher than in the exposed  
371 groups (IS and Cu1  $p<0.05$ ). For the soft tissues of *R. exoculata*, from Rainbow vent,  
372 LPO was of the same order of magnitude ( $0.3 \pm 0.05$  nmol.mg<sup>-1</sup> prot; Gonzalez-Rey et  
373 al. 2008). These results are 3-fold higher than that obtained for the coastal shrimps *P.*  
374 *elegans* and *P. varians*. (Gonzalez-Rey et al. 2008). Nevertheless, results suggest that  
375 Cu, at the exposure concentrations used in this study, is not inducing lipid peroxidation  
376 in *R. exoculata*.

377

378

### 379 **3.5. Relationship between metal accumulation and biomarkers responses**

380 The present results reinforce the fact that vent fauna, especially *R. exoculata*,  
381 have developed physiological adaptive strategies to face the metal-rich habitat they live

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

382 in. Moreover, the establishment of protection through the antioxidant enzymes activities  
383 seems correlated with the decreasing trend of LPO levels in the gills. The activity of  
384 enzymes seems to have a beneficial effect in decreasing the amount of ROS in cells and  
385 consequently limit the attack of ROS on membrane lipids. This result is in favour of a  
386 good detoxification process in the presence of copper. Nevertheless, the rather low  
387 impact of 4  $\mu\text{M}$  of Cu exposure on the shrimps might be due to the fact that they may  
388 have developed protective strategies as they are continuously bathed in a metal rich  
389 medium. Several studies have suggested the possibility of detoxification through the  
390 bacteria present in the gills chamber or in the gut (e.g. Jan et al. 2015). Part of the toxic  
391 compounds could be taken up by the bacteria hosted in the gills chamber of the shrimps  
392 and decreasing the amount of toxic compounds accumulated in shrimps tissues  
393 (Compère et al. 2002, Zbinden and Cambon-Bonavita 2003).

394         Given the small differences observed between the *in situ* and control groups for  
395 the variables assessed, the results suggest that the shrimps are apparently tolerant to the  
396 change of pressure (occurring during the water renewal) for at least the period of 72 h.  
397 The proposed protocol, including the pressurization and depressurization steps of the  
398 high-pressure aquarium for seawater renewal, seems to be an adequate method to assess  
399 toxicity to contaminants in deep-sea species. This may enable future longer term assays  
400 under high-pressure and low-temperature conditions, which will be more realistic from  
401 the perspective of potential deep-sea mining impacts. Nevertheless, future studies  
402 should attempt to increase the samples size and also look at the effects of other  
403 contaminants likely to be released during mining activities.

#### 404 405 **4. Conclusions**

406         This study gives a first appraisal of the amounts of metals, MTs, antioxidant  
407 enzymes and LPO in different tissues of the vent shrimp *Rimicaris exoculata* from the  
408 TAG hydrothermal vent field. *R. exoculata* shows a background protection against  
409 metal toxicity as the activities of enzymes were detected for the *in situ* group. A tissue  
410 specific response was observed for the biomarkers analysed. Shrimps appear to develop  
411 protection against exposure to Cu, with increasing activity of antioxidant enzymes  
412 (CAT and GST) and induction of MT in the higher Cu concentration exposure (4  $\mu\text{M}$ ).  
413 The ubiquity of the shrimp *R. exoculata* along the Mid-Atlantic Ridge and the similar

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

414 responses observed in other vent sites (e.g. Rainbow) with those obtained in this study  
415 render this species as a good model for a possible monitoring of mining effects in  
416 hydrothermal vent fauna. Moreover, results suggest that the use of the pressurized  
417 aquarium do not affect the shrimps in terms of biomarkers responses to metal exposure.  
418 This method looks promising for further biomarkers responses studies with other deep-  
419 sea fauna.

420

421

## 422 **Acknowledgements**

423 This work was developed under the MIDAS project (Managing impacts of deep-sea  
424 resource exploitation), funded by the European Commission 7th Framework  
425 Programme under the theme “Sustainable management of Europe’s deep sea and sub-  
426 seafloor resources” (Grant Agreement 603418). We thank the captain and the crew of  
427 the *Pourquoi pas?* Vessel and ROV Victor 6000 and the chief scientist of the BICOSE  
428 cruise. MA was supported by a MER MSc grant and the French mobility grants Idex  
429 and Aquimob.

430

431

## 432 **References**

- 433 Ahearn, G.A., Mandal, P.K., Mandal, A., (2004). Mechanisms of heavy-metal  
434 sequestration and detoxification in crustaceans: a review. *Journal of Comparative*  
435 *Physiology B* 174, 439–452.
- 436 Allen, C.E., Copley, J.T., Tyler, P.A., (2001). Lipid partitioning in the hydrothermal  
437 vent shrimp *Rimicaris exoculata*. *Marine Ecology* 22, 241–253.
- 438 Anderson, L., Halary, S., Lechaire, J.-P., Boudier, T., Frébourg, G., Marco, S., Zbinden,  
439 M., Gaill, F., (2008). Tomography of bacteria–mineral associations within the  
440 deep-sea hydrothermal vent shrimp *Rimicaris exoculata*. *Comptes Rendus Chimie*  
441 11, 268–280.
- 442 Bebianno, M.J., Company, R., Serafim, A., Camus, L., Cosson, R.P., Fiala-Medioni, A.,  
443 (2005). Antioxidant systems and lipid peroxidation in *Bathymodiolus azoricus*  
444 from Mid-Atlantic Ridge hydrothermal vent fields. *Aquatic Toxicology* 75, 354–  
445 373.

- 1 446 Bebianno, M.J., Langston, W.J., (1989). Quantification of metallothioneins in marine  
2 447 invertebrates using differential pulse polarography. *Portugaliae Electrochimica*  
3 448 *Acta* 7, 511–524.
- 4 449 Bradford, M.M., (1976). A rapid and sensitive method for the quantification of  
5 450 microgram quantities of protein utilizing the principle of protein-dye binding.  
6 451 *Analytical Biochemistry* 72, 248–254.
- 7 452 Campana, O., Taylor, A.M., Blasco, J., Maher, W.A., Simpson, S.L., (2015).  
8 453 Importance of subcellular metal partitioning and kinetics to predicting sublethal  
9 454 effects of copper in two deposit-feeding organisms. *Environmental Science and*  
10 455 *Technology* 49, 1806–14.
- 11 456 Company, R., Serafim, A., Cosson, R.P., Fiala-Médioni, A., Camus, L., Colaço, A.,  
12 457 Serrão-Santos, R., Bebianno, M.J., (2008). Antioxidant biochemical responses to  
13 458 long-term copper exposure in *Bathymodiolus azoricus* from Menez-Gwen  
14 459 hydrothermal vent. *Science of the Total Environment* 389, 407–417.
- 15 460 Company, R., Serafim, A., Cosson, R., Fiala-Médioni, A., Dixon, D. R., Bebianno,  
16 461 M.J., (2007). Adaptation of the antioxidant defence system in hydrothermal-vent  
17 462 mussels (*Bathymodiolus azoricus*) transplanted between two Mid-Atlantic Ridge  
18 463 sites. *Marine Ecology* 28, 93–99.
- 19 464 Company, R., Serafim, A., Cosson, R., Camus, L., Shillito, B., Fiala-Medioni, A.,  
20 465 Bebianno, M.J., (2006a). The effect of cadmium on antioxidant responses and the  
21 466 susceptibility to oxidative stress in the hydrothermal vent mussel *Bathymodiolus*  
22 467 *azoricus*. *Marine Biology* 148, 817–825.
- 23 468 Company, R., Serafim, A., Cosson, R., Fiala-Médioni, A., Dixon, D., Bebianno, M.J.,  
24 469 (2006b). Temporal variation in the antioxidant defence system and lipid  
25 470 peroxidation in the gills and mantle of hydrothermal vent mussel *Bathymodiolus*  
26 471 *azoricus*. *Deep Sea Research Part I: Oceanographic Research Papers* 53, 1101–  
27 472 1116.
- 28 473 Company, R., Serafim, A., Bebianno, M.J., Cosson, R., Shillito, B., and Fiala-Medioni,  
29 474 A., (2004). Effect of cadmium, copper and mercury on antioxidant enzyme  
30 475 activities and lipid peroxidation in the gills of the hydrothermal vent mussel  
31 476 *Bathymodiolus azoricus*. *Marine Environmental Research* 58, 377–381.
- 32 477 Compère, P., Martinez, A.-S., Charmantier-Daures, M., Toullec, J.-Y., Goffinet, G.,  
33 478 Gaill, F., (2002). Does sulphide detoxication occur in the gills of the hydrothermal  
34 479 vent shrimp, *Rimicaris exoculata*? *Comptes Rendus Biologies* 325, 591–596.
- 35 480 Copley, J.T.P., Jorgensen, P.B.K., Sohn, R.A., (2007). Assessment of decadal-scale  
36 481 ecological change at a deep Mid-Atlantic hydrothermal vent and reproductive time-  
37 482 series in the shrimp *Rimicaris exoculata*. *Journal of the Marine Biological*  
38 483 *Association of the United Kingdom* 87, 859–867.
- 39 484 Corbari, L., Zbinden, M., Cambon-Bonavita, M., Gaill, F., Compère, P., (2008).  
40 485 Bacterial symbionts and mineral deposits in the branchial chamber of the



- 1 486 hydrothermal vent shrimp *Rimicaris exoculata*: relationship to moult cycle.  
2 487 Aquatic Biology 1, 225–238.
- 3 488 Cosson, R., Vivier, J., (1997). Interactions of metallic elements and organisms within  
4 489 hydrothermal vents. Cahiers de Biologie Marine 43–50.
- 5  
6  
7 490 Cottin, D., Shillito, B., Chertemps, T., Tanguy, A., Léger, N., Ravaux, J., (2010).  
8 491 Identification of differentially expressed genes in the hydrothermal vent shrimp  
9 492 *Rimicaris exoculata* exposed to heat stress. Marine Genomics 3, 71–78.
- 10  
11  
12 493 Desbruyères, D., Almeida, A., Biscoito, M., Comtet, T., Kripounoff, A., Le Bris, N.,  
13 494 Sarradin, P.-M., Segonzac, M., (2000). A review of the distribution of  
14 495 hydrothermal vent communities along the Northern Mid-Atlantic Ridge: Dispersal  
15 496 vs environmental controls. Hydrobiologia 440, 201-216.
- 16  
17  
18 497 Depledge, M.H., Rainbow, P.S., (1990). Models of regulation and accumulation of trace  
19 498 metals in marine invertebrates. Comparative Biochemistry and Physiology Part C:  
20 499 Comparative Pharmacology 97, 1-7.
- 21  
22  
23 500 Di Giulio, R.T., Benson, W.H., Sanders B.M., Van Veld P.A., (1995) Biochemical  
24 501 mechanisms: Metabolism, adaptation and toxicity. In: Fundamentals of Aquatic  
25 502 Toxicology 2nd, Ed. G.M. Rand. Taylor & Francis, Washington. pp. 523-561.
- 26  
27  
28 503 Douville, E., Charlou, J.L., Oelkers, E.H., Bienvu, P., Jove Colon, C.F., Donval, J.P.,  
29 504 Fouquet, Y., Prieur, D., Appriou, P., (2002) The Rainbow vent fluids (36 14' N,  
30 505 MAR): the influence of ultramafic rocks and phase separation on trace metal  
31 506 content in Mid-Atlantic Ridge hydrothermal fluids. Chemical Geology 184, 37–48.
- 32  
33  
34 507 Edmonds, H.N., German, C.R., Green, D.R.H., Huh, Y., Gamo, T., Edmond, J.M.,  
35 508 (1996). Continuation of the hydrothermal fluid chemistry time series at TAG, and  
36 509 the effects of ODP drilling. Geophysical Research Letter 23, 3487–3489.
- 37  
38  
39 510 Engel D., Brouwer M., (1989). Metallothionein and metallothionein-like proteins.  
40 511 Physiological importance. In Advances in Comparative and Environmental  
41 512 Physiology Vol.5, Chapt.3, pp. 53-75, Springer, Berlin.
- 42  
43  
44 513 Erdelmeier, I., Gerard-Monnier, D., Yadan, J. C., Acudiere, J., (1998). Reactions of  
45 514 Nmethyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals.  
46 515 Mechanistic aspects of the colorimetric assay of lipid peroxidation. Chemical  
47 516 Research Toxicology 11, 1184–1194.
- 48  
49  
50 517 Flohe L., Gunzler W.A., (1984) Assay of glutathione peroxidase. Methods in  
51 518 Enzymology 105, 114-121.
- 52  
53  
54 519 Gaetke, L.M., Chow, C.K., (2003). Copper toxicity, oxidative stress and antioxidant  
55 520 nutrients. Toxicology 189, 147–63.
- 56  
57  
58 521 Gebruk, A.V., Pimenov, N.V., Savvichev, A.S., (1993). Feeding specialization of  
59 522 bresiliid shrimps in the TAG site hydrothermal community. Marine Ecology  
60 523 Progress Series 98, 247–253.
- 61  
62  
63  
64  
65

- 524 Gena, K., (2013). Deep sea mining of submarine hydrothermal deposits and its possible  
525 environmental impact in Manus Basin, Papua New Guinea. *Procedia Earth and*  
526 *Planetary Science* 6, 226–233.
- 527 Geret, F., Riso, R., Sarradin, P.-M., Caprais, J.-C., Cosson, R.P., (2002). Metal  
528 bioaccumulation and storage forms in the shrimp, *Rimicaris exoculata*, from the  
529 Rainbow hydrothermal field (Mid-Atlantic Ridge); preliminary approach to the  
530 fluid-organism relationship. *Cahiers de Biologie Marine* 43, 43–52.
- 531 Gonzalez-Rey, M., Serafim, A., Company, R., Gomes, T., Bebianno, M.J., (2008).  
532 Detoxification mechanisms in shrimp: Comparative approach between  
533 hydrothermal vent fields and estuarine environments. *Marine Environmental*  
534 *Research* 66, 35–37.
- 535 Gonzalez-Rey, M., Serafim, A., Company, R., Bebianno, M.J., (2007). Adaptation to  
536 metal toxicity: a comparison of hydrothermal vent and coastal shrimps. *Marine*  
537 *Ecology* 28, 100–107.
- 538 Greco, M.A., Hrab, D.I., Magner, W., Kosman, D.J., (1990). Cu, Zn superoxide  
539 dismutase and copper deprivation and toxicity in *Saccharomyces cerevisiae*.  
540 *Journal of Bacteriology* 172, 317–325.
- 541 Greenwald, R.A., (1985). Handbook of methods for oxygen radical research. CRC  
542 Press, Boca Raton, FL, USA.
- 543 Habig, W.H., Pabst, M.J., Fleischner, G., Gatmaitan, Z., Arias, I.M., Jakoby, W.B.,  
544 (1974). The identity of glutathione S-transferase B with ligandin, a major binding  
545 protein of liver. *Proceedings of the National Academy of Sciences* 71, 3879–3882.
- 546 Hannington, M., Jamieson, J., Monecke, T., Petersen, S., Beaulieu, S., (2011). The  
547 abundance of seafloor massive sulfide deposits. *Geology* 39, 1155–1158.
- 548 Halliwell, B., Gutteridge, J., (1984). Oxygen toxicity, oxygen radicals, transition metals  
549 and disease. *Biochemistry Journal* 219, 1–14.
- 550 Jan, C., Petersen J., Werner J., Huang S., Teeling H., Glöckner F.O., Golyshina O.V.,  
551 Dubilier N., Golyshin P.N., Jebbar M., Cambon-Bonavita, M.A., (2014). The gill  
552 chamber epibiosis of deep-sea *Rimicaris exoculata* shrimp revisited by  
553 metagenomics and discovery of zetaproteobacterial epibionts. *Environmental*  
554 *Microbiology* 16, 9, 2723-2738.
- 555 Kádár, E., Costa V., Santos. R.S., (2006). Distribution of micro-essential (Fe, Cu, Zn)  
556 and toxic (Hg) metals in tissues of two nutritionally distinct hydrothermal shrimps.  
557 *Science of the Total Environment* 358, 143-150.
- 558 Li, M., Hu, C., Zhu, Q., Chen, L., Kong, Z., Liu, Z., (2006). Copper and zinc induction  
559 of lipid peroxidation and effects on antioxidant enzyme activities in the microalga  
560 *Pavlova viridis* (Prymnesiophyceae). *Chemosphere* 62, 565-572.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
- 561 McCord, J.M., Fridovich, I., (1969). Superoxide dismutase: an enzymic function for  
562 erythrocyte (hemocuprein). *Journal of Biological Chemistry* 244, 6049–6055.
- 563 Mestre, N.C., Calado, R., Soares, A.M.V.M., (2014). Exploitation of deep-sea  
564 resources: The urgent need to understand the role of high pressure in the toxicity of  
565 chemical pollutants to deep-sea organisms. *Environmental Pollution* 185, 369–371.
- 566 Oliveira, C., Almeida, J., Guilhermino, L., Soares, A.M.V.M., Gravato, C., (2012).  
567 Acute effects of deltamethrin on swimming velocity and biomarkers of the  
568 common prawn *Palaemon serratus*. *Aquatic Toxicology* 124-125, 209–216.
- 569 Ponsard, J., Cambon-Bonavita, M.-A., Zbinden, M., Lepoint, G., Joassin, A., Corbari,  
570 L., Shillito, B., Durand, L., Cuff-Gauchard, V., Compère, P., (2013). Inorganic  
571 carbon fixation by chemosynthetic ectosymbionts and nutritional transfers to the  
572 hydrothermal vent host-shrimp *Rimicaris exoculata*. *ISME J* 7, 96–109.
- 573 Pourang, N., Dennis, J.H., Ghourchian, H., (2004). Tissue distribution and redistribution  
574 of trace elements in shrimp species with the emphasis on the roles of  
575 metallothionein. *Ecotoxicology* 13, 519–533.
- 576 Ravaux, J., Gaill, F., Le Bris, N., Sarradin, P.-M., Jollivet, D., Shillito, B., (2003). Heat-  
577 shock response and temperature resistance in the deep-sea vent shrimp *Rimicaris*  
578 *exoculata*. *Journal of Experimental Biology* 206, 2345–2354.
- 579 Rainbow, P.S., (1998). Phylogeny of trace metal accumulation in crustaceans. *In*:  
580 Langston W.J. and Bebianno M.J. (Eds). *Metal metabolism in aquatic*  
581 *environments*. London, Chapman and Hall, pp. 285-319.
- 582 Rieley, G., Van Dover, C.L., Hedrick, D.B., Eglinton, G., (1999). Trophic ecology of  
583 *Rimicaris exoculata*: a combined lipid abundance/stable isotope approach. *Marine*  
584 *Biology* 133, 495–499.
- 585 Roesijadi, G., (1992). Metallothioneins in metal regulation and toxicity in aquatic  
586 animals. *Aquatic Toxicology* 22, 81–114.
- 587 Schmidt, C., Corbari, L., Gaill, F., Le Bris, N., (2009). Biotic and abiotic controls on  
588 iron oxyhydroxide formation in the gill chamber of the hydrothermal vent shrimp  
589 *Rimicaris exoculata*. *Geobiology* 7, 454–464.
- 590 Schmidt, C., Le Bris, N., Gaill, F., (2008). Interactions of deep-sea vent invertebrates  
591 with their environment: the case of *Rimicaris exoculata*. *Journal of Shellfish*  
592 *Research* 27, 79–90.
- 593 Shillito, B., Gaill, F., Ravaux, J., (2014). The IPOCAMP pressure incubator for deep-  
594 sea fauna. *Journal of Marine Science and Technology* 22, 97–102.
- 595 Shillito, B., Hamel, G., Duchi, C., Cottin, D., Sarrazin, J., Sarradin, P.M., Ravaux, J.,  
596 Gaill, F., (2008). Live capture of megafauna from 2300 m depth, using a newly-  
597 designed pressurised recovery device. *Deep-Sea Research I* 55, 881–889.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
- 598 Silva, C., Oliveira, C., Gravato, C., Almeida, J.R., (2013). Behaviour and biomarkers as  
599 tools to assess the acute toxicity of benzo(a)pyrene in the common prawn  
600 *Palaemon serratus*. Marine Environmental Research 90, 39–46.
- 601 Simpson, S., Angel, B., Hamilton, I., Spadaro, D., Binet, M., (2008). Appendix 7, Water  
602 and Sediment Characterisation and Toxicity Assessment for the Solwara 1 Project  
603 Environmental Impact Statement in Coffey Natural Systems. Solwara 1 Project  
604 Environmental Impact Statement  
605 [[http://www.nautilusminerals.com/irm/content/environment-](http://www.nautilusminerals.com/irm/content/environment-reports2.aspx?RID=413)  
606 [reports2.aspx?RID=413](http://www.nautilusminerals.com/irm/content/environment-reports2.aspx?RID=413)]
- 607 Van Dover, C.L., (2014). Impacts of anthropogenic disturbances at deep-sea  
608 hydrothermal vent ecosystems: A review. Marine Environmental Research 102,  
609 59–72.
- 610 Van Dover, C.L., Fry, B., Grassle, J.F., Humphris, S., Rona, P.A., (1988). Feeding  
611 biology of the shrimp *Rimicaris exoculata* at hydrothermal vents on the Mid-  
612 Atlantic Ridge. Marine Biology 98, 209–216.
- 613 Vereshchaka, A., (1997). Comparative morphological studies on four populations of the  
614 shrimp *Rimicaris exoculata* from the Mid-Atlantic Ridge. Deep-Sea Research I 44,  
615 1905–1921.
- 616 Viarengo, A., Burlando, B., Cavaletto, M., Marchi, B., Ponzano, E., Blasco, J., (1999).  
617 Role of metallothionein against oxidative stress in the mussel *Mytilus*  
618 *galloprovincialis*. American Journal of Physiology-Regulatory, Integrative and  
619 Comparative Physiology 277, R1612–R1619.
- 620 Viarengo, A., Nott, J., (1993). Mechanisms of heavy metal cation homeostasis in marine  
621 invertebrates. Comparative Biochemistry and Physiology 104C, 355–372.
- 622 Vinagre, C., Madeira, D., Mendonça, V., Dias, M., Roma, J., Diniz, M.S., (2014). Effect  
623 of temperature in multiple biomarkers of oxidative stress in coastal shrimp. Journal  
624 of Thermal Biology 41, 38–42.
- 625 White, S., Rainbow, P., (1985). On the metabolic requirements for Copper and Zinc in  
626 Molluscs and Crustaceans. Marine Environmental Research 16, 215–229.
- 627 White, S., Rainbow, P., (1982). Regulation and accumulation of copper, zinc and  
628 cadmium by the shrimp *Palaemon elegans*. Marine Ecology Progress Series 8,  
629 101.
- 630 Zbinden, M., Shillito, B., Le Bris, N., de Villardi de Montlaur, C., Roussel, E., Guyot,  
631 F., Gaill, F., Cambon-Bonavita, M.-A., (2008). New insights on the metabolic  
632 diversity among the epibiotic microbial community of the hydrothermal shrimp  
633 *Rimicaris exoculata*. Journal of Experimental Marine Biology and Ecology 359,  
634 131–140.

1 635 Zbinden, M., Le Bris, N., Gaill, F., Compère, P., (2004). Distribution of bacteria and  
2 636 associated minerals in the gill chamber of the vent shrimp *Rimicaris exoculata* and  
3 637 related biogeochemical processes. *Marine Ecology Progress Series* 284, 237–251.  
4  
5 638 Zbinden, M., Cambon-Bonavita, M.-A., (2003). Occurrence of Deferribacterales and  
6 639 Entomoplasmatales in the deep-sea Alvinocarid shrimp *Rimicaris exoculata* gut.  
7 640 *FEMS Microbiology Ecology* 46, 23–30.  
8  
9 641  
10  
11 642  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

643 **Figure captions**

644 **Figure 1.** Metals concentrations (mean  $\pm$  SD) in tissues of *Rimicaris exoculata* for the  
645 different treatments. Values followed by the same letter are not significantly different ( $p$   
646  $> 0.05$ ) with capital letters for comparison of one tissue between groups and lowercase  
647 letters for comparison among one group. Gills (grey), Hepatopancreas (black),  
648 Abdominal muscle (white), IS: In situ group, C: Control, Cu1: exposed to 0.4 $\mu$ M, Cu2:  
649 exposed to 4 $\mu$ M.

650

651 **Figure 2.** Metallothioneins (MTs, mean  $\pm$  SD) in tissues of *Rimicaris exoculata* for the  
652 different treatments. Values followed by the same letter are not significantly different ( $p$   
653  $> 0.05$ ) with capital letters for comparison of one tissue between groups and lowercase  
654 letters for comparison among one group. Gills (grey), Hepatopancreas (black),  
655 Abdominal muscle (white), IS: In situ group, C: Control, Cu1: exposed to 0.4 $\mu$ M, Cu2:  
656 exposed to 4 $\mu$ M.

657

658 **Figure 3.** Superoxide dismutase (SOD), catalase (CAT), total glutathione peroxidase  
659 (GPx) and glutathione-S-transferase (GST) activities (mean  $\pm$  SD) in tissues of  
660 *Rimicaris exoculata* for the different treatments. Values followed by the same letter are  
661 not significantly different ( $p > 0.05$ ) with capital letters for comparison of one tissue  
662 between groups and lowercase letters for comparison among one group. Gills (grey),  
663 Hepatopancreas (black), Abdominal muscle (white), IS: In situ group, C: Control, Cu1:  
664 exposed to 0.4 $\mu$ M, Cu2: exposed to 4 $\mu$ M. Given the small amount of tissue available  
665 for HP, CAT was not analysed. GPx activity was not detected in the abdominal muscle.

666

667 **Figure 4.** Lipid peroxidation (LPO, mean  $\pm$  SD) in tissues of *Rimicaris exoculata* for  
668 the different treatments. Values followed by the same letter are not significantly  
669 different ( $p>0.05$ ) with capital letters for comparison of one tissue between groups and  
670 lowercase letters for comparison among one group. Gills (grey), Hepatopancreas  
671 (black), Abdominal muscle (white), IS: In situ group, C: Control, Cu1: exposed to  
672 0.4 $\mu$ M, Cu2: exposed to 4 $\mu$ M.

673

674 **Table captions**

675 **Table 1.** Metal concentration (mean  $\pm$  SD) in tissues of *Rimicaris exoculata* and  
676 *Mirocaris fortunata*. Data expressed in  $\mu\text{g.g}^{-1}$  d.w. or  $\mu\text{mol.g}^{-1}$  w.w.. G: Gills; H:  
677 Hepatopancreas; M: Abdominal muscle; S.T.: Soft tissue.

678

679 **Table 2.** Antioxidant enzymes activities (SOD, CAT, GPx, GST) (mean  $\pm$  SD) in  
680 tissues of the vent shrimp *Rimicaris exoculata* and coastal shrimps *Palaemon elegans*,  
681 *Palaemon serratus*, *Palaemon varians*. G: Gills; H: Hepatopancreas; M: Abdominal  
682 muscle; S.T.: Soft tissue.

683

684 **Supplementary Material**

685 **Table A.1.** Metal concentration per tissues (gills, hepatopancreas, muscle) and weighted  
686 mean (soft tissue) for *Rimicaris exoculata* in the different treatments (mean  $\pm$  SD),  
687 expressed in  $\mu\text{g.g}^{-1}$  d.w.. Values followed by the same letter are not significantly  
688 different ( $p>0.05$ ) with capital letters for comparison of one tissue between groups and  
689 lowercase letters for comparison among one group (for each metal). IS: In situ group; C:  
690 Control; Cu1: exposed to  $0.4\mu\text{M}$ ; Cu2: exposed to  $4\mu\text{M}$ .

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49

691 **Table 1.**

Species	Sites	Tissues	Cu	Cd	Co	Fe	Mn	Zn	References
<i>Rimicaris exoculata</i>	<b>TAG</b> ( $\mu\text{g}\cdot\text{g}^{-1}$ d.w.)	G	535 ± 205	1.24 ± 0.91	3 ± 2	6201 ± 4299	26 ± 11	277 ± 123	this study
		H	1562 ± 592	8.32 ± 1.68	3.7 ± 1.7	6122 ± 1764	29 ± 10	94 ± 64	
		M	98 ± 23	0.15 ± 0.03	0.3 ± 0.1	1052 ± 307	6 ± 2	88 ± 39	
		S.T.	379 ± 146	1.3 ± 0.4	1.6 ± 0.3	2740 ± 1191	16 ± 3.5	112 ± 31	
	(μmol.g <sup>-1</sup> w.w.)	G	0.75 ± 0.18	0.32 10 <sup>-3</sup> ± 0.1 10 <sup>-3</sup>	3.1 10 <sup>-3</sup> ± 5 10 <sup>-4</sup>	3.6 ± 1.1	0.022 ± 0.004	0.22 ± 0.06	this study
		H	2.51 ± 0.78	8.710 <sup>-3</sup> ± 2.1 10 <sup>-3</sup>	5.6 10 <sup>-3</sup> ± 2 10 <sup>-4</sup>	12.9 ± 2.6	0.06 ± 0.02	0.24 ± 0.09	
		M	0.11 ± 0.02	0.1 10 <sup>-3</sup> ± 0.03 10 <sup>-3</sup>	0.5 10 <sup>-3</sup> ± 1 10 <sup>-4</sup>	1.5 ± 0.4	0.008 ± 0.002	0.13 ± 0.02	
		S.T.	0.32 ± 0.18	0.0008 ± 0.0003	0.002 ± 0.0016	2.8 ± 1.5	0.02 ± 0.01	0.13 ± 0.04	
	<b>Rainbow</b> (μmol.g <sup>-1</sup> w.w.)	G	1.2 ± 0.6	0.014 ± 4 10 <sup>-3</sup>	-	25.5 ± 11.4	0.316 ± 0.18	17.9 ± 5.0	Geret et al. 2002
		H	0.61 ± 0.21	3.5 10 <sup>-3</sup> ± 9 10 <sup>-4</sup>	-	24.6 ± 1.8	0.076 ± 0.009	5.7 ± 1.7	
		M	0.26 ± 0.08	1.1 10 <sup>-3</sup> ± 9 10 <sup>-4</sup>	-	3.1 ± 1.86	0.039 ± 0.026	1.5 ± 1.1	
	<b>Rainbow</b> (μg.g <sup>-1</sup> d.w.)	G	1060 ± 200	-	-	90460 ± 48600	-	2900 ± 400	Kádár et al. 2006
H		700 ± 200	-	-	6640 ± 2230	-	800 ± 700		
M		300 ± 160	-	-	610 ± 260	-	300 ± 100		
<b>Rainbow</b> (μg.g <sup>-1</sup> d.w.)	S.T.	176.7 ± 74	0.49 ± 0.02	-	2122 ± 867	43.2 ± 10.4	209 ± 111	Gonzalez-Rey et al. 2008	
<i>Mirocaris fortunata</i>	<b>Rainbow</b> (μg.g <sup>-1</sup> d.w.)	G	1700 ± 500	-	-	122970 ± 41600	-	4300 ± 800	Kádár et al. 2006
		H	400 ± 100	-	-	3010 ± 620	-	600 ± 100	
		M	200 ± 60	-	-	940 ± 240	-	200 ± 40	
	<b>Lucky Strike</b> (μg.g <sup>-1</sup> d.w.)	G	1400 ± 500	-	-	44170 ± 15320	-	1900 ± 800	Kádár et al. 2006
		H	500 ± 200	-	-	1870 ± 250	-	900 ± 600	
		M	40 ± 10	-	-	190 ± 80	-	60 ± 30	

692



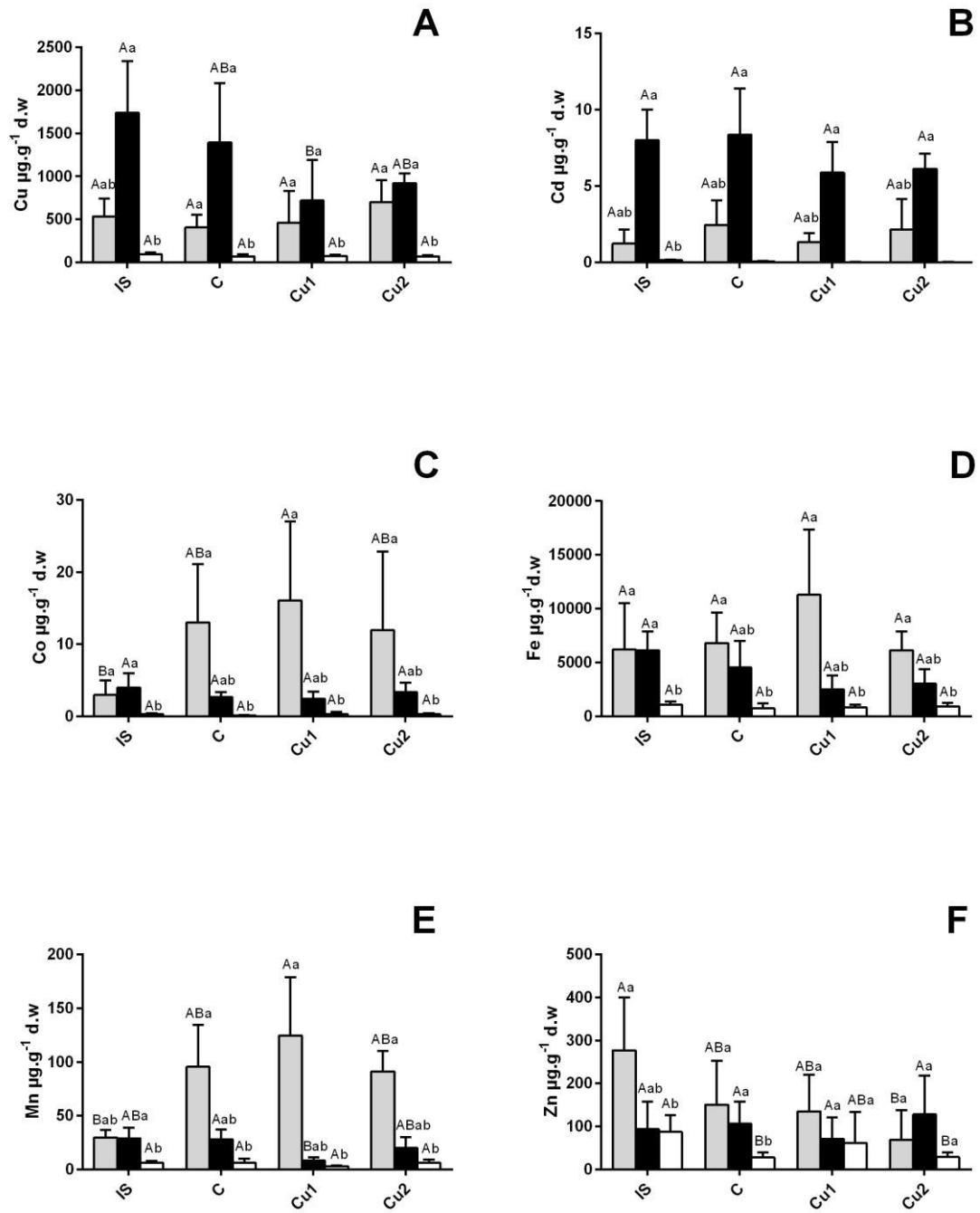
1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49

693 **Table 2.**

Species		SOD (U.mg <sup>-1</sup> .prot)	CAT (μmol.min.mg <sup>-1</sup> .prot)	GPx (nmol.min.mg <sup>-1</sup> .prot)	GST (nmol.min.mg <sup>-1</sup> .prot)	References
<i>R. exoculata</i>	G	53.3 ± 8.8	1.3 ± 0.5	191.9 ± 68.8	116.3 ± 52.7	this study
	H	95.3 ± 26.2	-	804.2 ± 167.7	225.6 ± 40.8	
	M	19.2 ± 5.1	0.6 ± 0.2	-	12.5 ± 2.2	
	S.T	1.9 ± 0.4	4.2 ± 0.3	10 ± 1	-	
<i>P. elegans</i>	M	-	5.3 ± 0.3*	-	0.56 ± 0.3	Vinagre et.al 2014
	S.T	3.9 ± 1.3	1.2 ± 0.3	15 ± 3	-	Gonzalez-Rey et al. 2008
<i>P. serratus</i>	M	-	0.7 ± 0.3*	-	0.3 ± 0.1	Vinagre et.al 2014
	H	-	1.5 ± 0.1	8.8 ± 0.6	3.1 ± 0.3	Silva et al. 2013
	H	-	1.5 ± 0.3*	4.7 ± 0.3	6.4 ± 0.4	Oliveira et al.2012
<i>P. varians</i>	S.T	4.4 ± 1.1	2 ± 0.4	22 ± 6	-	Gonzalez-Rey et al. 2008

694 \* nmol.min.mg<sup>-1</sup>.prot

695 **Figure 1**



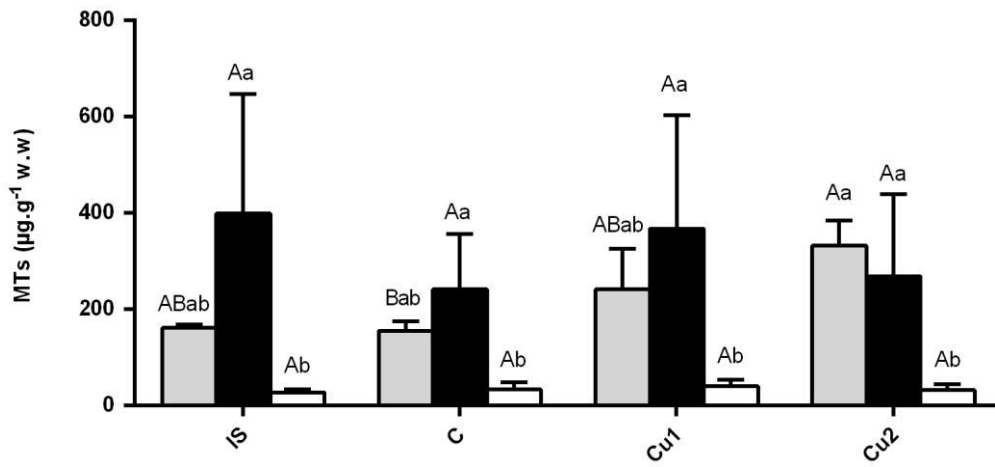
696

697

698

699

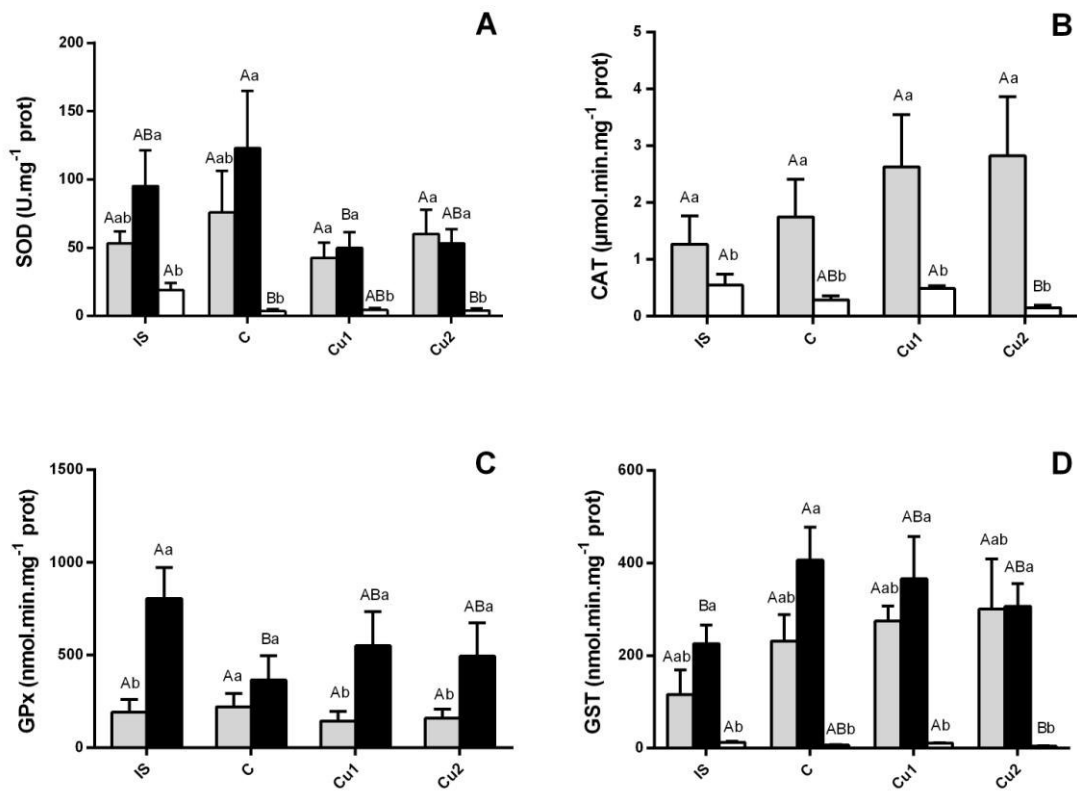
700 **Figure 2**



701

702

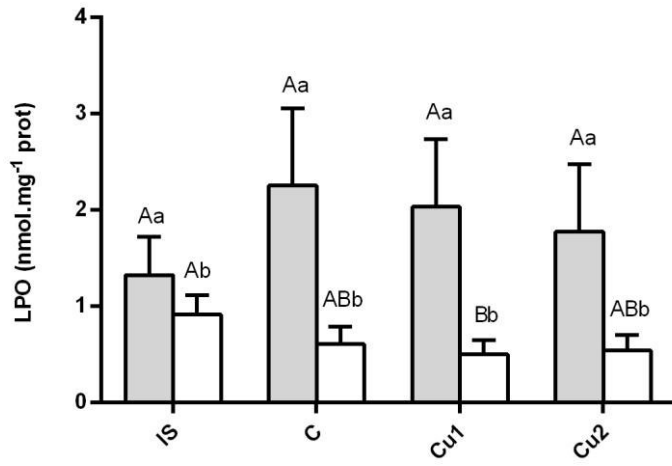
703 **Figure 3**



704

705

706 **Figure 4**



707

708

709

710

711

712

713

714

715

716 **Table A.1.**

		<b>IS</b>	<b>C</b>	<b>Cu1</b>	<b>Cu2</b>
<b>Gills</b>	Cu	535 ± 205 <sup>Aab</sup>	408 ± 145 <sup>Aa</sup>	461 ± 369 <sup>Aa</sup>	700 ± 258 <sup>Aa</sup>
	Cd	1 ± 0.9 <sup>Aab</sup>	2 ± 1.6 <sup>Aab</sup>	1 ± 0.6 <sup>Aab</sup>	2.2 ± 2 <sup>Aab</sup>
	Co	3 ± 2 <sup>Ba</sup>	13 ± 8 <sup>ABa</sup>	16 ± 11 <sup>Aa</sup>	12 ± 10.9 <sup>ABa</sup>
	Fe	6201 ± 4299 <sup>Aa</sup>	6791 ± 2851 <sup>Aa</sup>	11285 ± 6051 <sup>Aa</sup>	6129 ± 1751 <sup>Aa</sup>
	Mn	30 ± 7 <sup>Bab</sup>	96 ± 39 <sup>ABa</sup>	125 ± 54 <sup>Aa</sup>	91 ± 19 <sup>ABa</sup>
	Zn	277 ± 123 <sup>Aa</sup>	151 ± 102 <sup>ABa</sup>	135 ± 85 <sup>ABa</sup>	69 ± 69 <sup>Ba</sup>
	<b>Hepato-pancreas</b>	Cu	1740 ± 600 <sup>Aa</sup>	1395 ± 690 <sup>ABa</sup>	719 ± 474 <sup>Ba</sup>
Cd		8 ± 2 <sup>Aa</sup>	8 ± 3 <sup>Aa</sup>	6 ± 2 <sup>Aa</sup>	6 ± 1 <sup>Aa</sup>
Co		3.7 ± 2 <sup>Aa</sup>	2.7 ± 0.7 <sup>Aab</sup>	2 ± 0.9 <sup>Aab</sup>	3.4 ± 1.3 <sup>Aab</sup>
Fe		6122 ± 1764 <sup>Aa</sup>	4541 ± 2466 <sup>Aab</sup>	2488 ± 1297 <sup>Aab</sup>	3046 ± 1331 <sup>Aab</sup>
Mn		29 ± 10 <sup>ABa</sup>	28 ± 9 <sup>Aab</sup>	8 ± 3 <sup>Bab</sup>	20 ± 10 <sup>ABab</sup>
Zn		94 ± 64 <sup>Aab</sup>	107 ± 51 <sup>Aa</sup>	72 ± 50 <sup>Aa</sup>	128 ± 90 <sup>Aa</sup>
<b>Muscle</b>		Cu	94 ± 22 <sup>Ab</sup>	71 ± 24 <sup>Ab</sup>	75 ± 17 <sup>Ab</sup>
	Cd	0.15 ± 0.03 <sup>Ab</sup>	0.06 ± 0.05 <sup>ABb</sup>	0.02 ± 0.02 <sup>Bb</sup>	0.01 ± 0.03 <sup>Bb</sup>
	Co	0.34 ± 0.1 <sup>Ab</sup>	0.13 ± 0.09 <sup>Ab</sup>	0.34 ± 0.26 <sup>Ab</sup>	0.3 ± 0.1 <sup>Ab</sup>
	Fe	1052 ± 307 <sup>Ab</sup>	724 ± 490 <sup>Ab</sup>	813 ± 245 <sup>Ab</sup>	906 ± 349 <sup>Ab</sup>
	Mn	6.3 ± 1.6 <sup>Ab</sup>	6.2 ± 4 <sup>Ab</sup>	3 ± 0.8 <sup>Ab</sup>	6.5 ± 2.6 <sup>Ab</sup>
	Zn	88 ± 39 <sup>Ab</sup>	28 ± 12 <sup>Bb</sup>	62 ± 72 <sup>ABa</sup>	29 ± 11 <sup>Ba</sup>
	<b>Soft tissue</b>	Cu	379 ± 146 <sup>A</sup>	331 ± 154 <sup>A</sup>	265 ± 98 <sup>A</sup>
Cd		1.3 ± 0.4 <sup>A</sup>	1.4 ± 0.3 <sup>A</sup>	1.3 ± 0.45 <sup>A</sup>	1.5 ± 0.7 <sup>A</sup>
Co		1.1 ± 0.3 <sup>A</sup>	3.4 ± 2 <sup>A</sup>	3.5 ± 1.7 <sup>A</sup>	2.9 ± 1.5 <sup>A</sup>
Fe		2740 ± 1191 <sup>A</sup>	2342 ± 1041 <sup>A</sup>	3757 ± 1547 <sup>A</sup>	2340 ± 810 <sup>A</sup>
Mn		11 ± 3.4 <sup>A</sup>	30 ± 20 <sup>A</sup>	30 ± 22 <sup>A</sup>	32 ± 15 <sup>A</sup>
Zn		112 ± 31 <sup>A</sup>	63 ± 29 <sup>AB</sup>	85 ± 53 <sup>AB</sup>	49 ± 14 <sup>B</sup>

717

718

719

720

721

722

723

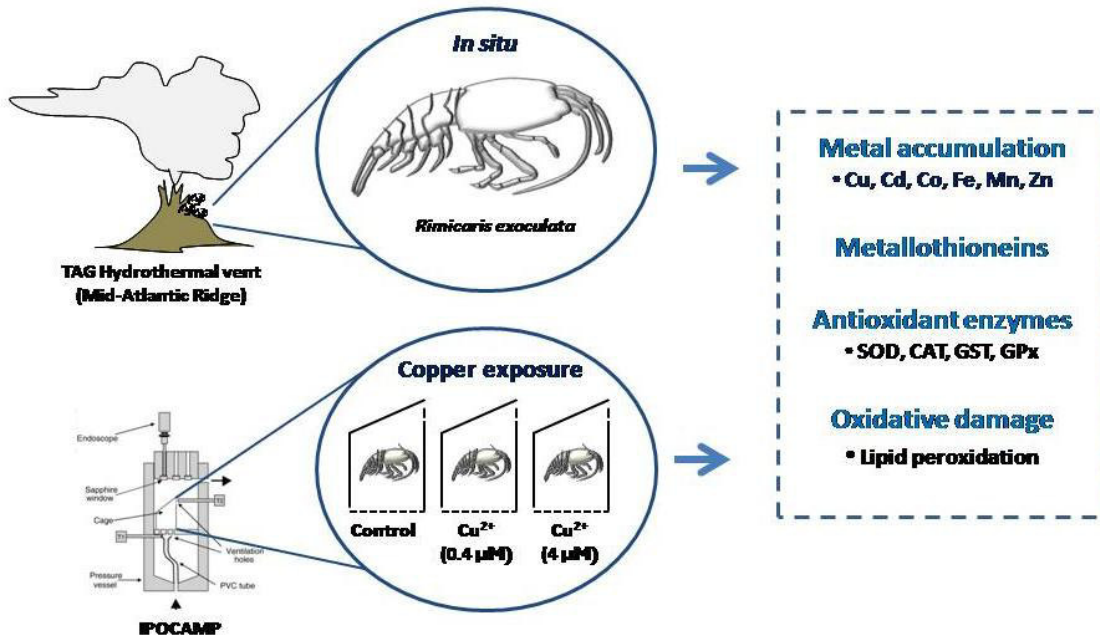
724

725

726

727

728 Graphical Abstract



729

730

1 **Highlights**

2 • *Rimicaris exoculata* have developed antioxidant defences to cope with a metal-  
3 rich habitat.

4 • The depressurization of the test chamber every 12h did not affect the biomarkers  
5 response.

6 • The proposed method seems useful to assess toxicity to contaminants in deep-  
7 sea species.