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**Detoxification mechanisms in shrimp: comparative approach  
between hydrothermal vent fields and estuarine environments**

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**Abstract**

Hydrothermal vents are extreme deep-sea habitats that, due to their singular features, still intrigue scientific communities. Swift growth rates and profuse biomass of biological communities can be observed, despite of their inherently unstable physical-chemical and toxic conditions, indicating that organisms inhabiting this environment must be well adapted to these inhospitable conditions. The caridean shrimp, *Chorocaris chacei*, *Mirocaris fortunata* and *Rimicaris exoculata*, together with bathymodiolid mussels, dominate the vent fauna along the Mid-Atlantic Ridge (MAR). Crustacean species are widely used as biological indicators of environmental alterations, since they play a key ecological role as planktivorous grazers, epibenthic scavengers or as prey species. The biological consequences of the hydrothermal metal-rich environment in shrimp species are still largely unknown. Therefore, the aim of this study was the determination of the metal levels (Ag, Cd, Cu, Fe, Mn and Zn), metallothioneins (MT)

and lipid peroxidation (LPO) in shrimp species collected in Rainbow, Lucky Strike and Menez-Gwen vent sites, in order to evaluate their different adaptation strategies toward metals when compared with two common coastal shrimp species (*Palaemon elegans* and *Palaemonetes varians*) from a fairly unpolluted estuarine system in south Portugal (Ria Formosa). Results show significant differences in metal concentrations, MT levels and lipid peroxidation between vent and coastal shrimp and also between shrimp species from the same site. This indicates that biochemical responses in both vent and coastal shrimp are affected not only by the environmental characteristics but also by inter-specific differences. Nevertheless, these responses apparently grant a successful adaptation for the survival in a metal-extreme environment.

*Keywords:* Metallothionein; Lipid Peroxidation; Metals; Hydrothermal vents; Shrimp

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Metals can be extremely toxic to organisms when present at high levels. Nonetheless, metal-enriched hydrothermal vent fields: Rainbow (RB) (36°13'N, 33°54'W, 2300m), Lucky-Strike (LS) (37°17'N, 32°16'W, 1700 m) and Menez-Gwen (MGw) (37°51'N, 31°31'W, 850 m) manage, despite of their geochemical and physical distinct settings (Douville et al., 2002), to simultaneously comprise high metal concentrations with prolific biological communities. Trying to understand this apparent contradiction, metal concentrations (Ag, Cd, Cu, Fe, Mn and Zn), metallothionein (MT) and lipid peroxidation (LPO) levels were measured and compared between three vent shrimp species: *Rimicaris exoculata* and *Mirocaris fortunata* collected from RB and *Chorocaris chacei* from both LS and MGw with two common coastal species from a fairly unpolluted estuarine system Ria Formosa (RFor) (37°03'N; 07°47'W) in South Portugal (Bebianno, 1995).

Hydrothermal vent species were collected using the remote operated vehicle VICTOR6000 during SEAHMA I cruise. Coastal shrimp were collected with a sub-superficial tow using a shrimp net (~40 cm diameter). Both vent and coastal species were immediately frozen (-80°C) and stored until analysis. Before dissection, shrimp were thoroughly cleaned with 0.02M TRIS-HCl buffer (pH 8.6), assuring minimal surface/particle contamination. Exoskeletons were separated from soft tissues; gills and hepatopancreas were not considered for analysis. MT analyses were performed in the soft tissues according to Bebianno and Langston (1989). Metal concentrations were determined in the soft tissues and exoskeleton by AAS. Lipid peroxidation was determined according to Erdelmeier et al. (1998). Total protein concentrations in supernatants were determined according to Lowry method (Lowry, 1951). Non-parametric test of Kruskal-Wallis ( $p < 0.05$ ) was used to compare statistical differences

among the variables and Tukey's test was used when significant differences between averages were observed.

The most accumulated metals were Fe, Zn, Mn and Cu while Ag and Cd were the less accumulated in all shrimp species (Fig. 1). The concentration of metals accumulated in different tissues of crustaceans vary widely between metals and between taxa (Rainbow, 1998) and is related to its speciation, function of tissues and other biotic variables (Pourang et al., 2004). In hydrothermal species, this tendency reflects the metal concentrations in the hydrothermal fluids. Metal concentrations for MAR hydrothermal vents ranged from: Ag (4.3-17 nM), Cd (9-12 nM), Cu (2  $\mu$ M), Fe (2-18  $\mu$ M), Mn (59-68  $\mu$ M) and Zn (2  $\mu$ M) in Menez-Gwen; Ag (4.7-25 nM), Cd (18-79 nM), Cu (2 - 30  $\mu$ M), Fe (70-920  $\mu$ M), Mn (77-450  $\mu$ M) and Zn (2 - 40  $\mu$ M) in Lucky Strike and Ag (47 nM), Cd (130 nM), Cu (140  $\mu$ M), Fe (24000  $\mu$ M), Mn (2250  $\mu$ M) and Zn (160  $\mu$ M) in Rainbow (Douville et al., 2002). In the Ria Formosa system metal concentrations ranged from: Cd (0.9 - 4.5 nM), Cu (0.02 - 0.05  $\mu$ M), Fe (8 - 52  $\mu$ M), Mn (2.5 - 6.3  $\mu$ M) and Zn (0.02 - 0.03  $\mu$ M) (Caetano et al., 1997). Since Fe, Zn and Cu are essential elements, high levels of these metals in shrimp tissues are attributed to their essentiality (Pourang et al., 2004). Accordingly, the low levels of Cd (a non-essential metal) in the tissues reflect the lower metal concentrations in the seawater where shrimp were collected (Géret et al., 2002a).

The measured metal concentrations vary with site, species and type of tissue, i.e. for hydrothermal shrimp, Fe is the most prominent metal, whilst for coastal shrimp is Mn. In *M. fortunata* from RB and *C. chacei* from LS the exoskeleton had significantly higher metal concentrations than the soft tissues (Fig. 1). Exoskeleton contains a significant proportion of metals, which is reduced prior to moulting. Moulting is an important aspect of crustacean physiology that influences metal concentrations and its

distribution between soft tissues and exoskeleton (Pourang et al., 2004). However, in *C. chacei* from MGw all metals except Mn occur in higher concentrations in the soft tissues when compared to the exoskeleton (Fig. 1). Since Mn can substitute calcium in calcium carbonate, this metal is predominately found in calcified parts. The carapace of decapods acts as a depository in which Mn can be accumulated (Pourang et al., 2004). In coastal shrimp metals occur in higher concentrations in soft tissues compared to exoskeleton. Metals are taken up by crustaceans in a form initially available to bind with metabolites in the receiving cell, with the potential to be transported elsewhere in the body via the haemolymph (Marsden and Rainbow 2004). Hence, significant differences in metal content between tissues reflect different bioavailability, function and efficiency in accumulating metals. Rainbow (1998) concluded that Zn body concentrations in decapods are regulated to approximately those required for metabolic demand with relatively little Zn stored in detoxified form. However this seems not to be the case especially in hydrothermal vent species.

Like metal levels, MT concentrations also varied between species. MT levels in *C. chacei* from LS were four-fold higher than those for the same species from MGw (Table 1) and significantly higher than other vent or coastal shrimp species. *C. chacei* collected from MGw may reflect a more recent exposure to metals, contrarily to what occurred in shrimp from LS. Therefore, it seems that metals were not yet remobilized from the soft tissues to the exoskeleton, in order to be detoxified by MT. This protein does not respond immediately to the metal exposure as observed in laboratory studies (Géret et al., 2002b) and this may explain the significant differences observed between the levels of metals and MT in *C. chacei* from these two different hydrothermal vents.

However, LPO levels in *C. chacei* were significantly lower than those of *R. exoculata* and *M. fortunata* from RB and similar to the coastal shrimp (Table 1).

Moreover, positive correlations exist between MT levels and Ag (*P. elegans* - MT =  $2.14 + 0.71 \text{ Ag}$ ,  $r = 0.997$ ), Zn (*P. varians* - MT =  $1.03 + 1.32 \text{ Zn}$ ,  $r = 0.998$ ) and Cd (*C. chacei* from LS - MT =  $-43.33 + 112.9 \text{ Cd}$ ,  $r = 0.989$ ). Since, no crustacean regulates the body concentration of non-essential metals (Rainbow, 1998); MT may have a role in metal detoxification in the coastal species. Although no significant differences in MT levels between the two shrimp species were found, *M. fortunata* had four-fold higher LPO levels than *P. varians* ( $p < 0.05$ ). Considering that RB is the vent site with higher metal concentrations, basal levels of LPO in native shrimp may be naturally elevated. Therefore, LPO levels point out that shrimp from RB are exposed to more significant stressful environmental conditions when compared to their coastal counterparts. Nevertheless, a negative correlation between antioxidant enzymes and MT levels was detected in vent species (Gonzalez-Rey et al., 2007). Hence, MT (particularly for *C. chacei* from LS) may have an antioxidant role in ROS detoxification inside the cells, mainly in the elimination of hydroxyl radical, as described by Chubatsu and Meneghini (1993). Finally, the results suggest that biochemical responses in vent and coastal shrimp are not exclusively affected by environmental characteristics (i.e. microhabitats) but also by interspecific differences (i.e. feeding habits and moulting cycles), and only a complex combination of metal detoxification systems grant a successful adaptation to survival in metal-rich environments.

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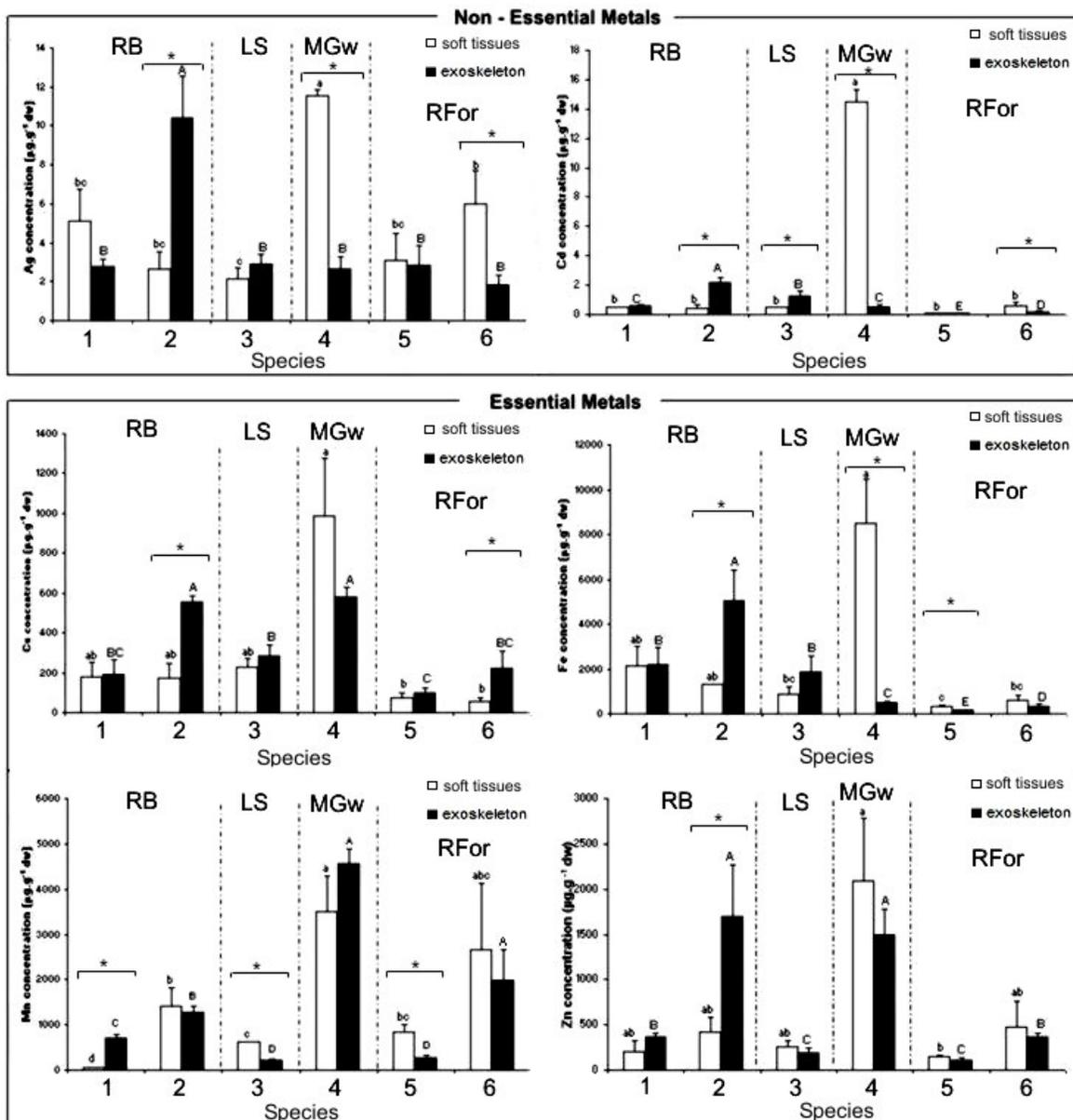
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**Figure Caption**

Fig. 1. Non-essential (Ag and Cd) and essential metal concentrations (Cu, Fe, Mn and Zn) ( $\mu\text{g. g}^{-1}$  dry weight) in soft and exoskeleton tissues of shrimp species. *R. exoculata* (1), *M. fortunata* (2) and *C. chacei* (3,4) from Mid- Atlantic hydrothermal vent fields and *P. elegans* (5) and *P. varians* (6) from Ria Formosa coastal area (Portugal). The data represent average and  $\pm$  standard deviation (SD) for n=24. Values followed by the same lower case letter represent a non statistical difference between soft tissues for each metal and values followed by the same upper case letter represent a non statistical difference between exoskeleton tissues for each metal ( $p > 0.05$ ). \* Significant differences between soft tissues and exoskeleton for each species within every metal ( $p < 0.05$ ).

Figure 1



**Table 1.**

Metallothionein concentrations (MT) and lipid peroxidation levels (LPO) in soft tissues of five shrimp species: *R. exoculata*, *M. fortunata* and *C. chacei* from Mid- Atlantic hydrothermal vent fields and *P. elegans* and *P. varians* from Ria Formosa coastal area (Portugal). The data represent average and  $\pm$  standard deviation (SD) for n= 24. Values followed by the same letter are not statistically different ( $p > 0.05$ )

Sample Location		Species	MT mg.g <sup>-1</sup> prot	LPO nmol.g <sup>-1</sup> prot
Hydrothermal Vents	Rainbow	<i>Rimicaris exoculata</i>	7.19 $\pm$ 0.75 <sup>b*</sup>	306.04 $\pm$ 50.00 <sup>A</sup>
		<i>Mirocaris fortunata</i>	1.27 $\pm$ 0.19 <sup>d*</sup>	369.17 $\pm$ 36.39 <sup>A</sup>
	Lucky Strike	<i>Chorocaris chacei</i>	11.69 $\pm$ 1.23 <sup>a</sup>	107.33 $\pm$ 34.17 <sup>B</sup>
	Menez-Gwen	<i>Chorocaris chacei</i>	2.74 $\pm$ 1.81 <sup>cd</sup>	n.d.
Coastal Area	Ria Formosa	<i>Palaemons elegans</i>	4.34 $\pm$ 1.00 <sup>c*</sup>	77.45 $\pm$ 8.50 <sup>B</sup>
		<i>Palaemonetes varians</i>	1.65 $\pm$ 0.39 <sup>d*</sup>	84.68 $\pm$ 25.48 <sup>B</sup>

\* Adapted from Gonzalez-Rey et al., 2007

n.d. not determined