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Metals and detoxification mechanisms in shrimp: comparative approach between hydrothermal vent fields and estuarine environments

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Chapter 1.

“It is not the strongest of the species that survives, nor the most intelligent. It is the one that is the most adaptable to change”

- Charles Darwin

1. Introduction

Even though three decades have passed since their discovery, on an expedition to the Galapagos volcanic Rift off the coast of Ecuador (Von Damm *et al.*, 1998; Desbruyères *et al.*, 2000, 2001), hydrothermal vents still raise an enormous interest from the scientific community (Vereshchaka, 1997; Tyler & Young, 2003; Van Dover & Lutz, 2004).

For the first time, a productive ecosystem almost independent of solar energy was unveiled (Priuer *et al*., 1997). The hydrothermal vent environment on the deep sea is a highly specialized and fundamentally different compared with the photic zone of the ocean (Larsen *et al.*, 1997). After all, odd is intriguing. It seems that, regardless of all the apparent inhospitable display of features presented there (namely, the presence of high metal and dissolved gases concentrations), a profuse “alien-like” biological community proliferate in these extreme environments (Van Dover & Fry, 1994; Fowler & Tunnicliffe, 1997; Prieur *et al.*, 1997; Poltz *et al.*, 1998; Horikoshi *et al.*, 1998; Vereshchaka & Vinogranov, 1999; Dixon *et al.*, 2000; Desbruyères *et al.*, 2000, 2001; Little & Vrijenhoek, 2003; Hardivillier *et al.*, 2004; Minic *et al.*, 2006; Company *et al.*, 2007).

At hydrothermal vents the duration of exposure to potential toxic compounds is on a geological timescale which provides a unique historical perspective on environmental contamination and species adaptation (Dixon *et al.*, 2000). Since, anthropogenic pollution by comparison is a very recent occurrence (<100 years) it is licit to relate hydrothermal vent environment as a natural equivalent phenomenon (Geret *et al*., 2002; Martins *et al*., 2001; Ruelas-Inzunza *et al*., 2003) and hydrothermal vent communities as powerful laboratory tools to assess the impact of industry on world ecosystems (Kádár *et al.*, 2006a).

Survival of the hydrothermal inhabiting species, particularly crustaceans, implies many modifications of their biochemical components (eg. proteins and enzymes, membranes and nucleic acids) as well as other physiological adaptations (Hardivillier *et al.*, 2004). In an ecotoxicological perspective, it is particularly relevant the comprehension of the effective maintenance systems to counter act the toxic effects of metals and other chemical compounds.

2. Contrasting Environments

2.1. Mid-Ocean Ridges (MOR)

Mid-ocean ridges are sea-floor spreading centres with volcanic, tectonic, and hydrothermal activity. Extending more than 75,000 km around the globe, they are an almost continuous volcanic mountain chain situated at bathyal and abyssal depths (1500–4000 m) (Zekely *et al.*, 2006) with hydrothermal vents scattered along their length (Van Dover, 2000) (Figure 1). The occurrence, size, mineralogy and geologic setting of hydrothermal fields may be controlled by many variables, including crustal permeability, thickness and composition, magma supply, sea floor depth, spreading rate, and the spatial and temporal history of volcanic/tectonic activity (Langmuir *et al.*, 1997).

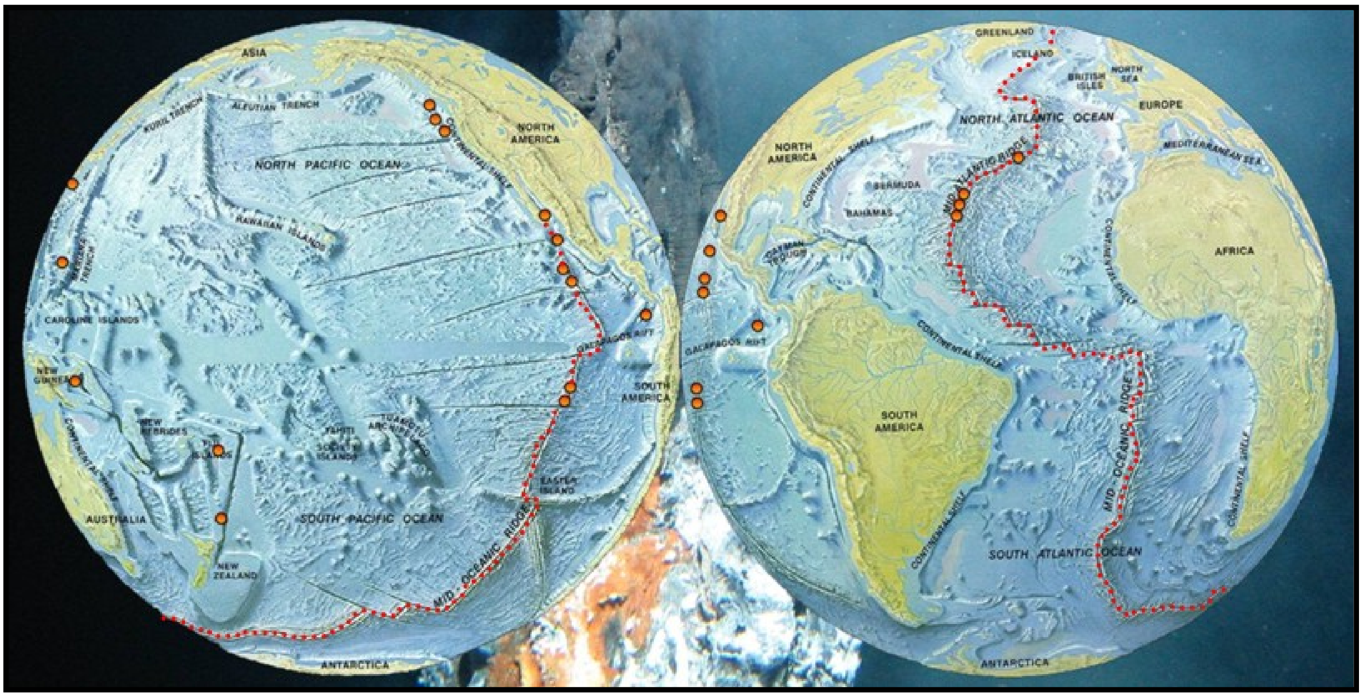

Figure 1. Geographic distribution of the known hydrothermal vent sites through out the Atlantic and Pacific Mid-Ocean Ridges (adapted from www.pbs.org/wgbh/nova/abyss/frontier/vent.html)

2.1.1. Hydrothermal vents

Hydrothermal systems are a widespread feature of the global mid-ocean ridge network, and represent expulsion of high-temperature fluid circulating through and cooling young oceanic crust (Von Damm, 1995). Generally, hydrothermal vents are deep-sea chimney-like structures characterized by an unique and inherently unstable physico-chemical properties such as elevated pressure (up to 420 atm), abrupt temperature (from 2-4 ◦C to 400 ◦C) and pH gradients, chemical toxicity vent emissions, anoxia and complete absence of light (Geret *et al.*, 1998; Desbruyères *et al.*, 2000, 2001; Geret *et al.*, 2002; Colaço *et al.*, 2006; Minic *et al.*, 2006; Zekely *et al.*, 2006).

Hydrothermal vent emissions exhibit natural high metal (both essential and non essential) concentrations (e.g. Ag, As, Cd, Cu, Fe, Mn, Zn, etc.) as a result of the interaction between seawater and magmatic rocks. Consequently, the hydrothermal vent fluids have a very high temperature (300–350ºC) and are naturally enriched in silica (SiO_2) and dissolved gases (e.g. H_2S, CH_4, H_2, CO_2) (Von Damm, 1990, 1992; Lowell *et al.*, 1995; Von Damm *et al.*, 1995; Sarradin *et al.*, 1998, 1999; Douville *et al.*, 2002).

The highly productive communities inhabiting deep-sea hydrothermal vents all rely upon chemo-autotrophic microbiological production. These microorganisms are at the base of this photo-synthesis-independent trophic web and derive their energy from the oxidation of reduced compounds emitted by hydrothermal venting (Childress & Fisher, 1992; Chevaldonné & Godfroy, 1997). Vent communities contain remarkable taxonomic novelty at the specific and supraspecific level (e.g. new families, orders and classes), of the 442 species so far discovered, only 9% are known to occur elsewhere (Fowler & Tunnicliffe, 1997; Little & Vrijenhoek, 2003). Although most of the biodiversity at hydrothermal vents can be linked to taxonomic groups that include inconspicuous individuals (e.g. polychaete worms, nematodes, gastropods and copepod crustaceans), most of the biomass is formed by a few visually striking species. Including vestimentiferan tube worms (Siboglinidae), vent mussels (Bathymodiolinae), vent clams (Vesicomyidae) and vent shrimp (Alvinocarididae), all of which harbour chemoautotrophic bacterial symbionts (Vereshchaka, 1997; Van Dover 2000).

2.1.2. Mid-Atlantic Ridge (MAR)

The Mid-Atlantic Ridge (MAR) is an oceanic rift that separates the North American Plate, from the Eurasian Plate in the North Atlantic, and the South

American Plate, from the African Plate in the South Atlantic. Numerous hydrothermal vent fields have been identified on the MAR (Table I), notably in the Azores Triple Junction region (36º-38ºN), which include: Menez-Gwen, Lucky Strike and Rainbow. Their main characteristics are further described (sections a, b, c).

Table I. Location of vent fields observed on the M. A. R. between 11ºN and 38ºN (Desbruyères, 2000)

Vent field	Latitude N	Longitude W	Depth	References
Logatchev	14º45´	44º 58.7´	2930 – 3020	Gebruk *et al.* (1997)
Snake Pit	23º23´	44º56.1´	3480	Segonzac (1992)
T.A.G.	26º08´	44º49.6´	3635 – 3670	Rona *et al.* (1993) Van Dover (1995)
Broken Spur	29º10´	43º10.4´	3050 – 3875	Van Dover (1995) Murton *et al.* (1995)
Rainbow	36º13´	33º54.1´	2260 – 2350	Fouquet *et al.* (1997)
Mount Saldanha	36º33´	33º28.0´	2300	
Lucky Strike	37º17´	32º16.3´	1620 – 1730	Langmuir *et al*. (1993) Van Dover *et al*. (1996)
Menez-Gwen	37º51´	31º31.2´	840 – 865	Fouquet *et al.* (1997) Fouquet *et al.* (1999)

a) Menez-Gwen (37°51'N, 31°31´W, 850 m)

Menez-Gwen is situated in the volcanic segment between 37º35´N and 38ºN discovered during the French cruise DIVA I in 1994 (Fouquet *et al.*, 1997,1999; Desbruyères *et al.*, 2000; Charlou *et al.*, 2000; Desbruyères *et al.*, 2001). The ridge segment is characterized by a 17 km diameter and 700 m high volcano at its centre. This volcano is split by an along-axis, 2 km-wide and 300 m deep graben (Fouquet *et al.*, 1997; Radford-Knoery *et al.*, 1998; Barriga, 1999; Fouquet *et al.*, 1999; Charlou *et al.*, 2000; Desbruyères *et al.*, 2000, 2001). This site, covering a 200 m^2 area between 871 and 847 m depths, is very young and active. Young chimneys, typically less than 5 m high, are growing on the fresh pillows and are essentially composed of white anhydrite formed by heating seawater with hot and clear hydrothermal fluids (T= 281ºC) (Barriga, 1999; Charlou *et al.*, 2000). Around these white chimneys, colonized only by scarce hydrothermal fauna, diffuse hot waters are observed discharging from small and flat mounds enriched in barite (Charlou *et al.*, 2000). In the limit between lava and the anhydrite deposits, there are important mussel' colonies (e.g. *Bathymodiolus azoricus*). Extensive bacterial mats cover some of these populations. On the active deposits (chimney walls) and amongst mussels, important populations of shrimp *Chorocaris chacei* and *Mirocaris fortunata* are found (Desbruyères *et al.*, 2001).

Menez-Gwen vents present temperatures between 271°C and 284°C with pH between 4.2 and 4.8 (Douville *et al.*, 2002). The hydrothermal fluids in Menez-Gwen have low H_2S concentrations when compared to other hydrothermal fields (< 1.5 mM). The metal concentration in this hydrothermal vent site, even though clearly higher than in average Ria Formosa and sea water, is relatively lower compared to the other MAR vent sites (Table II)

b) Lucky Strike (37°17'N, 32°16'W, 1700 m)

Discovered in 1992, during the joint US-French FAZAR expedition (FAZAR Scientific Team, 1993), Lucky Strike is one of the largest known active fields in the modern ocean (Langmuir *et al.*, 1997; Desbruyères *et al.*, 2001). While navigating a dredge, a piece of sulphide with live vent organisms (mussels) was recovered — hence the name ‘Lucky Strike’ (Von Damm *et al.*, 1998). A striking feature of the Lucky Strike segment is the prominent seamount in the centre of the rift valley that forms a broad platform with an area of ~50 km^2 and maximum relief above the rift valley floor of ~250-300 m (Langmuir *et al.*, 1997). The venting area consists of a large central lava lake surrounded by the summits of three volcanic cones (Fouquet *et al.*, 1997, Khripounoff *et al.*, 2000, Desbruyères *et al.*, 2001). As observed for Menez-Gwen, the dominant species is the mussel *B. azoricus.* Dense mats of filamentous bacteria covered certain areas of mussel beds. On the walls of small active diffusers, several tens of very active shrimp populations of *Chorocaris chacei* are observed (Desbruyères *et al.*, 2001).

Although deeper than Menez-Gwen vent field, this hydrothermal vent site is relatively shallow (1700 m) (Van Dover *et al.*, 1996). Lucky Strike presents a high hydrothermal fluid range of temperatures (185 - 324ºC) and pH (3.4 – 5.0) when compared to the other sampling sites (Charlou *et al.*, 2000; Desbruyères *et al.*, 2001). However, Lucky Strike and Menez-Gwen are frequently associated regarding vent fluid characteristics, since both are basalt-hosted sites strongly affected by recent volcanic activity and shallow circulation systems. Major metal concentrations in Lucky Strike are presented in Table II (Douville *et al.*, 2002).

c) Rainbow (36°13'N, 33°54'W, 2300m)

The Rainbow hydrothermal field was discovered in 1997 at the north-eastern corner of the South AMAR (Alvin Mid-Atlantic Ridge) segment (Fouquet *et al.*, 1997). Aside of being the deepest vent field (2270 – 2300 m) (Fouquet *et al.*, 1997; Desbruyères *et al.*, 2001; Douville *et al.*, 2002) of the three hydrothermal vent fields present in this study, is mostly hosted in serpentinised ultramafic rocks exposed by cross cutting faults (Chavagnac *et al.*, 2005) which implies that this vent field is tectonically controlled (Douville *et al.*, 2002). Active chimneys, emitting hot-temperature fluids, are current in an area of 15 km^2, arranged longitudinally along a 200 m wide stretch of ridge (Fouquet *et al.*, 1997; Douville *et al.*, 2002). Shrimp species, especially *R. exoculata* and *M. fortunata* are present in great abundance at all active sites of the field, along with the mussel species *B. azoricus* (Desbruyères *et al.*, 2001).

Compared to Menez-Gwen and Lucky Strike typically basalt-hosted vent-fluids, Rainbow fluids have a very uniform composition showing evidence of phase separation at depth (Charlou *et al.*, 2002; Douville *et al.*, 2002) and present unique features such as, the lowest end-member pH (~2.8), the highest chloride concentration (750 mM), temperature (365ºC) and metal concentrations in the MAR hydrothermal area. Particularly Fe concentration (24 mM), which is quite a few fold higher than the other sampling sites (Table II) (Douville *et al.*, 2002).

2.2. Coastal Lagoon System

Littoral ecosystems are interfaces between the open ocean and land ecosystems, often, typified as being important habitats for a wide variety of species and comprising highly productive communities (Santos *et al.*, 2004).

2.2.1. Ria Formosa (37º03´ N; 07º47´ W)

The Ria Formosa is a highly-dynamic and productive mesotidal lagoon, with a surface area of approximately 16,300 ha, separated from the ocean by a system of five sand barrier islands and six inlets, which extend for about 55 km along the south coast of Portugal (Bebianno, 1995; Caetano *et al.*, 2002; Santos *et al.*, 2004; Gamito & Erzini, 2005). In general, the lagoon is relatively shallow, consisting in large part of salt marsh and shallow channels (Falcão & Vale, 1998; Gamito & Erzini, 2005). The average water depth is <2 m and the tidal height varies from a maximum of 3.7 m at spring tide to a minimum of 0.4 m at neap tide (Santos *et al.*, 2004). Most of the water volume is drained in each tidal cycle, thereby imposing a short residence time and an intense exchange of materials between the Ria Formosa and the adjacent coastal waters (Bebianno, 1995; Caetano *et al.*, 2002; Santos *et al.*, 2004).

This area supports several economically important activities, such as tourism and particularly shellfish and fish aquaculture (Sprung, 1994, Geret *et al.*, 2003, Gamito & Erzini, 2005). Over the last decade, there has been a noticeable deterioration of the lagoon’s water quality. Reflecting the intense economic development allied with both poorly efficient water treatment (inexistent until the 90´s) and enormous population density variations. Thereby, the major inputs of pollutants, including trace metals (Table II), came from untreated sewage and domestic effluents from two cities (Faro and Olhão) (Bebianno, 1995; Padinha *et al.*, 2000, Caetano *et al.*, 2002).

Table II. Temperature, pH and concentration of chemical species in the end-member fluids of lagoon system Ria Formosa (South Portugal) (Caetano *et al*., 1997)and MAR vent fields (Menez-Gwen, Lucky Strike and Rainbow) compared with average seawater (Douville *et al*., 2002).

Site	Menez-Gwen 37°51'N, 31°31´W	Lucky Strike 37°17'N, 32°16'W	Rainbow 36°13'N, 33°54'W	Ria Formosa 37º03´ N, 07º47´ W	Seawater
T(°C)	271 - 284	185 - 324	365	17.3	-
pH	4.5	3.4 - 5.0	2.8	8.28	7.8
H_2S (mM)	1.5	0.6 - 3.4	1.0	n.d.	~0
CO_2(mM)	17-20	8.9 - 28	<16	n.d.	-
CH_4(mM)	1.35-2.63	0.5 - 0.97	2.2-2.5	n.d.	~0
Ag (nM)	4.3-17	4.7 - 25	47	n.d.	0.023
Cd (nM)	<9 - 12	18 - 79	130	0.9 – 4.5	0.7
Cl (mM)	380-400	413 - 554	750	n.d.	546
Co (µM)	<2	<2	13	n.d.	<2
Cu (µM)	<2	<2-30	140	0.02 – 0.05	0.0033
Fe (µM)	<2-18	70 - 920	24000	8 -52	0.0045
Mn (µM)	59 – 68	77 - 450	2250	2.5 – 6.3	0.0013
Ni (µM)	<2	<2	3	n.d.	<2
Si (mM)	8.2 – 11.2	8.2 - 10	6.9	n.d.	<0.2
Zn (µM)	<2	<2-40	160	0.02 – 0.03	0.028

n.d. – not determined

3. Sampled Species Description

Crustacean species are widely used as biological indicators of environmental alterations, since they play an ecological key role as planktivorous grazers, epibenthic scavengers or as prey species and simultaneously present high sensivity to metals (Clark, 1997).

3.1. Hydrothermal Vent Species

To date, more than 125 species representing 33 families of decapods have been reported from hydrothermal vents. All endemic vent shrimps are treated as members of the family Alvinocarididae. Currently recognized vent-associated species of shrimp belong to six genera: *Alvinocaris*, *Chorocaris*, *Mirocaris*, *Nautilocaris*, *Opaepele* and *Rimicaris* (for further detail see: Martin & Haney, 2005).

Shrimp species living on the MAR possess a range of morphological, anatomical and physiological adaptations to the hydrothermal environment, as well as different patterns of micro-distribution and feeding behaviour (Gebruk *et al.*, 2000).

3.1.1 Micro-distribution Patterns

Shrimp micro-scale distribution patterns depend on the habitat landscape at the various site (Gebruk *et al.*, 2000).

i) Rimicaris exoculata (Williams & Rona, 1986)

R. exoculata is perhaps the most extensively studied vent decapod species to date. Dense clusters (up to 3000 individuals per square meter) are observed in small depressions between chimneys expelling superheated sulphide-loaded fluid (Gebruk *et al.*, 1993; Segonzac *et al.*, 1993; Gebruk *et al.*, 2000; Polz *et al.*, 1998; Desbruyères *et al.*, 2001; Martin & Haney, 2005). The adult shrimps live permanently in or very close to variable and extreme environmental conditions such as high temperature, high sulphide and metal content, high level of carbon dioxide, low oxygen level, and low pH (Martinez *et al.*, 2005).

ii) Chorocaris chacei (Martin & Hessler 1990)

C. chacei was listed as rare/patchy but at the same time a dominant species on chimneys and walls at Lucky Strike and on chimney bases at Rainbow and Menez Gwen (Desbruyères *et al.*, 2000).

iii) Mirocaris fortunata (Martin & Christiansen, 1995)

M. fortunata appears to have the broadest range of any Alvinocarididae shrimp, occurring at seven vent sites along the MAR (Martin & Haney, 2005). This species are most abundant around sulphide diffusers and/or on chimneys covered by iron oxides, with higher densities in zones of high biomass, such as mussels beds (Desbruyères *et al.*, 2001).

3.1.2. Vent Shrimp Feeding Behaviour

R. exoculata and *C. chacei* are morphologically similar (Gebruk *et al.*, 1993, 2000); both develop epibiotic bacteria supporting structures, namely modified mouthparts and the inside of the carapace, during metamorphosis to the adult form (Gebruk *et al.*, 2000). The main food of adult *R. exoculata* is filamentous bacteria that grow on these structures. *C. chacei* in intermediary sizes feed on such bacteria, however on adult stage their feeding habits rely on scavenging and predation (Gebruk *et al.*, 2000). *M. fortunata* present an opportunist feeding behaviour, ingesting tissues of mussels, other shrimp and invertebrates when available (Gebruk *et al.*, 2000; Kádár *et al.*, 2006b, 2007).

3.2. Ria Formosa species

3.2.1. Distribution Patterns

i) Palaemon elegans (Rathke, 1837)

The intertidal shrimp *Palaemon elegans* is widespread along the Atlantic coast of Europe, in the North Sea, the Mediterranean and the western Baltic (Campbell, 1994). It is common in slightly brackish water close to river mouths, tidal rockpools, and in Zostera, Posidonia and Cymodocea sea grasses (González-Ortegón & Cuesta, 2006).

ii) P*alaemonetes varians* (Leach, 1814)

The brackish water shrimp, *Palaemonetes varians* is present in North European coasts down to the Mediterranean basin. Individuals of this species inhabit salt marsh areas, irrigation channels and inland coastal ponds, characterized by stagnant, highly turbid water, with broad seasonal variations in salinity and temperature. Hence they show a high tolerance of hypoxia (Aguzzi *et al.*, 2005; González-Ortegón & Cuesta, 2006)

3.2.2. Coastal Shrimp Feeding Behaviour

Both species represent significant components of the trophic web, since their feeding behaviour alters depending on the availability of a particular food. Therefore these species can act as detritivores, assimilating microflora and fauna; primary and secondary consumers, respectively feeding on epiphytic microalgae, or predating meiofauna and small infaunal polychaetes, oligochaetes, nematoides, molluscs and other crustaceans (Anderson, 1985; Janas *et al.*, 2004).

4. Metals in Aquatic Environments and Associated Toxicity

Metals are serious pollutants because they are stable compounds not readily removed by oxidation, precipitation or any other natural processes (Clark, 1997). They are common seawater and sediments components, which origin can be due to either natural (i.e. rock erosion, volcanic and hydrothermal activities) or anthropogenic actions (Mason & Jenkins, 1995). The problems associated with metal contamination were first highlighted in industrially advanced countries because of their larger untreated industrial discharges, and especially because of incidents of mercury and cadmium pollution in Sweden and Japan (Hossain & Khan, 2001).

The term 'heavy metal' is used synonymously with 'trace metal' and includes both essential and non-essential trace metals. Essential metals (i.e. Cu, Fe, Mn and Zn) are defined as elements whose lack causes vital metabolic alterations in organisms; however these effects are potentially reversible when normal metal levels are recovered. Still, after reaching certain threshold values their presence becomes toxic. Non-essential metals (i.e. Ag and Cd) beside of not having any known biological function have direct toxic or lethal effect at every low concentration (Mason & Jenkins, 1995; Rainbow, 2005).

Aquatic invertebrates are exposed to both essential and non-essential metals from both dissolved and particulate phases. Particulate metals can be accumulated by animals following ingestion and digestion of food, depending on the particular metal involved and its chemical form in the diet (Wang & Fisher, 1999). On the other hand dissolved metals will be taken up from solution through permeable body surfaces (Wang & Fisher, 1999; Rainbow, 2007).

4.1. Metals Uptake in Crustaceans

The concentration of metals accumulated in different tissues of crustaceans vary widely between metals and between taxa and is related to its speciation, function of tissues and other biotic variables (Rainbow, 1998, 2002; Pourang *et al.*, 2004; Rainbow 2005, 2007). As an important aspect of crustacean physiology, moulting may influence metal concentrations and the distribution between soft tissues and exoskeleton (Pourang *et al.*, 2004).

Concentration of metals within crustacean body will depend on (a) the level of bioavailability, (b) speciation of metal in seawater, and (c) the capacity of the organism to keep body concentration constant. For decapod crustaceans the free (hydrated) metal ion is commonly considered the available (toxic) form for uptake from solution. Any increase in total dissolved metal concentration would correspondingly increase the free metal ion concentration, and thereby lead to an increase in metal uptake rate (Rainbow, 1995). An insightful schematic representation of the body metal content, uptake and detoxification of a decapod crustacean is given below (Figure 2).

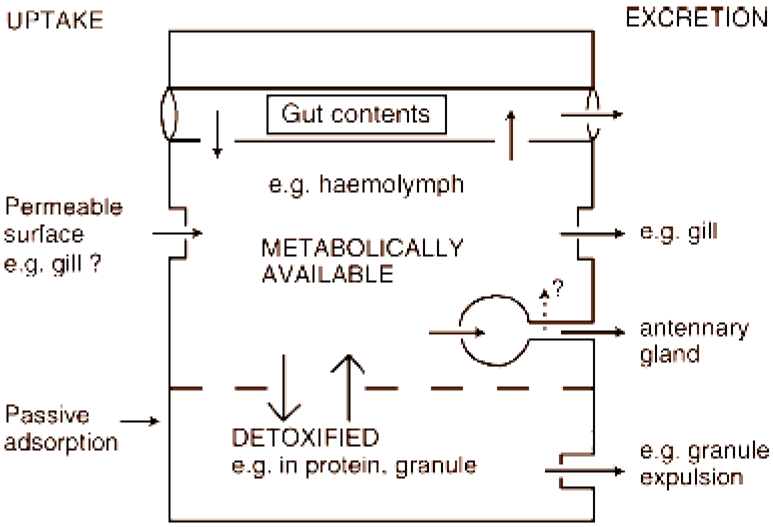

Figure 2. Schematic representation of the body metal content of a decapod crustacean (Rainbow, 1988).

Many studies have been made concerning metal accumulation in shrimp (namely in coastal species, i.e. Nugegoda & Rainbow, 1995; Bat *et al.*, 1999; Lorenzon *et al.*, 2000), but very few have been made in vent shrimp species (Geret *et al.*, 2002; Colaço *et al.*, 2006; Kádár *et al.*, 2006b, 2007).

5. Metal Detoxification Mechanisms

Resistance to metal toxicity involves several detoxification strategies, which keep the metal from interacting at sensitive sites in the organism (Viarengo & Nott, 1993). This is commonly achieved by two major mechanisms: metallic ions are either trapped in insoluble granules or bound to specific metalloproteins, such as the small cytosolic protein - metallothionein (Langston *et al.*, 1998; Dabrio *et al.*, 2002) whose synthesis is triggered by metals´ intracytoplasmic level (Viarengo & Nott, 1993; Mason & Jenkins, 1995).

The accumulation of metals also promotes the production of highly toxic radical oxygen species (ROS), inside cells and consequently the enhancement of lipid peroxidation in tissues (Fridovich, 1998) and eventual DNA damage inflictions (Pruski & Dixon, 2003). Aerobic organisms possess a baseline status of antioxidant systems involved in a variety of detoxification reactions, performed namely by antioxidant enzymes (such as, superoxide dismutase - SOD, catalase – CAT, selenium-dependent glutathione peroxidase - Se-GPx and total glutathione peroxidase – GPx) in order to assure the maintenance of pro-oxidant/antioxidant balance which is crucial for cellular homeostasis (Winston & Di Giulio, 1991; Lemaire & Livingstone, 1993; Livingstone, 2001).

5.1 Metallothionein

The first metallothionein (MT) was found in equine renal cortex by Margoshes & Vallee (1957). MTs are ubiquitous and inducible non-enzimatic proteins characterized by low molecular weight (Mr 6–8 kDa), high Cys content (20–30%) but no aromatic or His residues, heat stability. The thiol groups (–SH) of cysteine residues enable MTs to bind particular metals (Hg, Ag, Cu, Cd, Zn) (Stillman, 1995; Nordberg, 1998) and for that reason MTs are widely regarded as biomarkers of metal exposure, especially in the marine environment (Roesijadi, 1992; Amiard & Cosson, 1997; Langston *et al.*, 1998). The alignment of Cys-Cys, Cys-X-Cys and Cys-X-Y-Cys sequences where X and Y are amino acids other than cysteine, is the criterion that allows the distinction between different structural MT classes and that leads to many isoforms of the same protein (Langston *et al.*, 1998; Amiard *et al.*, 2006).

The biological functions of metallothioneins are still a subject of controversy, however they are usually regarded in the: (i) homeostasis of essential trace metals, such as Zn and Cu, (ii) detoxification of metals and protection from metal toxicity, (iii) free radical scavenging, (iv) cell growth and proliferation, (v) action of immunity response levels and (vi) protection against carcinogenic agents (Roesijadi, 1992; Viarengo & Nott, 1993; Mason & Jenkins, 1995; Nordberg, 1998; Park *et al.*, 2001; Chan *et al.*, 2002; Serafim, 2004).

Tissues directly involved in metal uptake, storage and excretion have a high capacity to synthesise MTs. In aquatic organisms, these proteins have mostly been identified in the digestive gland (also termed the midgut gland or hepatopancreas) and gills of molluscs and crustacean.

Crustaceans, in addition to metal excretion via molting are known for their high MT induction capacity (Cosson & Vivier, 1997). Amiard *et al.* (2006) recently compiled several studies showing a clear relationship between metallic concentrations and MT levels, and an evident induction of this protein after Cd, Cu and Zn exposures in aquatic invertebrates, including decapod shrimps. However, very few studies were made regarding MT levels and hydrothermal vent shrimp species (Geret *et al.*, 2002).

5.2 Antioxidant Enzymes vs. Oxidative Stress

Trace metals are known for their ability to enhance the cellular formation of free radicals or reactive oxygen species (ROS) which are responsible for the oxidative stress that leads to several cellular and metabolic alterations, including protein degrading or lipid peroxidation of membranes (Viarengo *et al.*, 1990, Fridovich, 1998, Viarengo *et al.*, 2000). Oxidative stress is generally defined as a disruption of the balance between the levels of oxidants (ROS) and reductants (antioxidants) in the organisms (Granot & Kohen, 2004). Most of them have acquired relevant protective mechanisms to maintain the lowest possible levels of ROS inside the cells. The protective mechanisms include both non-enzymatic (ascorbic acid, β-carotene, glutathione, and α-tocopherol) and enzymatic (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)) antioxidant systems (Liu *et al.*, 2006).

Superoxide dismutase (SOD) is one of the main antioxidant defence enzymes generated in response to oxidative stress. SOD detoxifies superoxide radicals by converting them to hydrogen peroxide and oxygen. The hydrogen peroxide, which can easily diffuse through the cell membranes into the cytoplasm, is then reduced to water and oxygen by catalase and glutathione peroxidases, resulting in innocuous compounds to the cell (Fridovich, 1995, 1998; Gómez-Anduro *et al.*, 2006). The activities of antioxidant enzymes were described for very few hydrothermal vent species, mainly in the mussels *Bathymodiolus azoricus* from MAR vent fields (Bebianno *et al.*, 2005, Company *et al.*, 2006).

5.2.1. Lipid Peroxidation

Lipids, especially those derived from polyunsaturated fatty acids, may react with ROS to form peroxyl adducts, which in turn react with other lipids in an autooxidant chain reaction (Armstrong & Browne, 1994; Girotti, 1998). Lipid peroxidation is a major source of many cytotoxic products such as aldehydes produced by the decomposition of hydroperoxides. Malondialdehyde (MDA) and 4-hydroxyalkenals (4-HDA) concentrations are used as indices of the oxidative breakdown of lipids in the membranes (Erdelmeier *et al.*, 1998), which can indicate if the integrity of the biological membranes is being assaulted or has been compromised (Duttie, 1993).

In addition, production of LPOs also indicates that levels of active oxygen species are overwhelming a number of different antioxidant enzymes, thus LPO is also an indicator of oxidative stress (Rilkans and Hornbrook, 1997; Girotti, 1998). Ultimately, LPO and consequent tissue damage are the major problems associated with failure of the antioxidant system (Erdelmeier *et al.*, 1998) and since the lipid composition reflects the interface between the cell and its environment, the perturbation in lipid composition may reflect subtle responses to environment pollution (Geret *et al.*, 2003).

6. Objective

Considering that metal concentrations on hydrothermal vents are extremely high regarding the average coastal and seawater, there was a particular interest to specifically address shrimp´ metal detoxification mechanisms as an adaptation strategy in contrasting environments. Therefore, one of the aims of this thesis was to compare metal (Ag, Cd, Cu, Fe, Mn and Zn) concentrations, metallothioneins (MTs) and lipid peroxidation (LPO) levels between three vent shrimp species: *Rimicaris exoculata* and *Mirocaris fortunata* collected from Rainbow vent field and *Chorocaris chacei* from both Lucky Strike and Menez-Gwen vent fields with two common coastal species (*Palaemon elegans* and *Palaemonetes varians*) from a fairly unpolluted estuarine system Ria Formosa in South Portugal in order to evaluate different adaptation strategies towards metals (Chapter 2).

As antioxidant enzymes can also protect against metal-induced reactive oxygen species. This thesis was the first attempt to compare MT levels and antioxidant defence systems from two shrimp species on the hydrothermal vent site where metal levels were higher (Rainbow) and coastal shrimp and try to explain the importance of such responses in the resistance and tolerance towards a metal-rich environment (Chapter 3).

All vent species were collected using the remote operated vehicle VICTOR6000 during the SEAHMA I cruise (July-29th to Agost-14th of 2002), carried out by SEAHMA project (Seafloor and Sub-Seafloor Hydrothermal Modelling in the Azores Sea – PDCTM/MAR/15281/1999).

All coastal species were collected from the margin of the Ria Formosa lagoon system with a sub-superficial tow using a shrimp net (~40 cm diameter).

Chapter 2

Comparison of metal (Ag, Cd, Cu, Fe, Mn and Zn) concentrations, metallothioneins and lipid peroxidation between vent and coastal shrimp species

Gonzalez-Rey M., Serafim A., Company R., Gomes T. & Bebianno M.J. Detoxification mechanisms in shrimp: comparative approach between hydrothermal vent fields and estuarine environments. Marine Environmental Research, *in press*

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 shrimp, *Chorocaris chacei*, *Mirocaris fortunata* and *Rimicaris exoculata*, dominate the vent fauna along the Mid-Atlantic Ridge. 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 coastal shrimp species (*Palaemon elegans* and *Palaemonetes varians*) from the estuarine system Ria Formosa (Portugal). 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, 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 an unpolluted estuarine system Ria Formosa (RFor) (37º03´N; 07º47´W) in South Portugal (Bebianno 1995).

Hydrothermal vent species were collected during SEAHMA I cruise. Coastal shrimp were collected with a sub-superficial tow using a shrimp net. Exoskeletons were separated from soft tissues; gills and hepatopancreas were not considered for analysis. MT analyses were performed by DPP in the soft tissues. Metal concentrations were determined in the soft tissues and exoskeleton by AAS. LPO was determined according to Erdelmeier et al. (1998). Non-parametric test of Kruskall-Wallis (p<0.05) was used as well as Tukey's test.

The most accumulated metals were Fe, Zn, Mn and Cu while Ag and Cd were the less accumulated in all shrimp species (Figure 1). The concentrations of metals accumulated in different tissues of crustaceans vary widely between metals and taxa and is related to its speciation and function of tissues (Pourang *et al.*, 2004). In hydrothermal species, this tendency reflects the metal concentrations in the hydrothermal fluids. 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 in the tissues reflect the lower metal concentrations in the seawater where shrimp were collected (Caetano et al., 1997; Douville et al., 2002; Géret et al., 2002a).

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 (Figure 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. However, in *C. chacei* from MGw all metals except Mn occur in higher concentrations in the soft tissues when compared to the exoskeleton (Figure 1). Since Mn can substitute Ca in $CaCO_3$, this metal is predominately found in calcified parts (Pourang et al., 2004). In coastal shrimp metals occur in higher concentrations in soft tissues. 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 & Rainbow 2004). Hence, significant differences in metal content between tissues reflect different bioavailability, function and efficiency in accumulating metals.

MT concentrations also varied between species. In *C. chacei* from LS, MT levels were four-fold higher than those from MGw (Table I) and significantly higher than other 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*.

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 I). Moreover, positive correlations exist between MT levels and Ag (*P. elegans* - MT = 2.14 + 0.71 Ag, *r* = 0.997), Zn (*P. varians* - MT = 1.03 + 1.32 Zn, *r* = 0.998) and Cd (*C. chacei* from LS - MT = -43.33 + 112.9 Cd, *r* = 0.989). Since no crustacean regulates the body concentration of non-essential metals (Pourang et al., 2004), 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, as described by Chubatsu & Meneghin (1993).

Finally, the results suggest that biochemical responses in vent and coastal shrimp are not exclusively affected by environmental characteristics but also by interspecific differences, and only a complex combination of metal detoxification systems grant a successful adaptation to survival in metal-rich environments.

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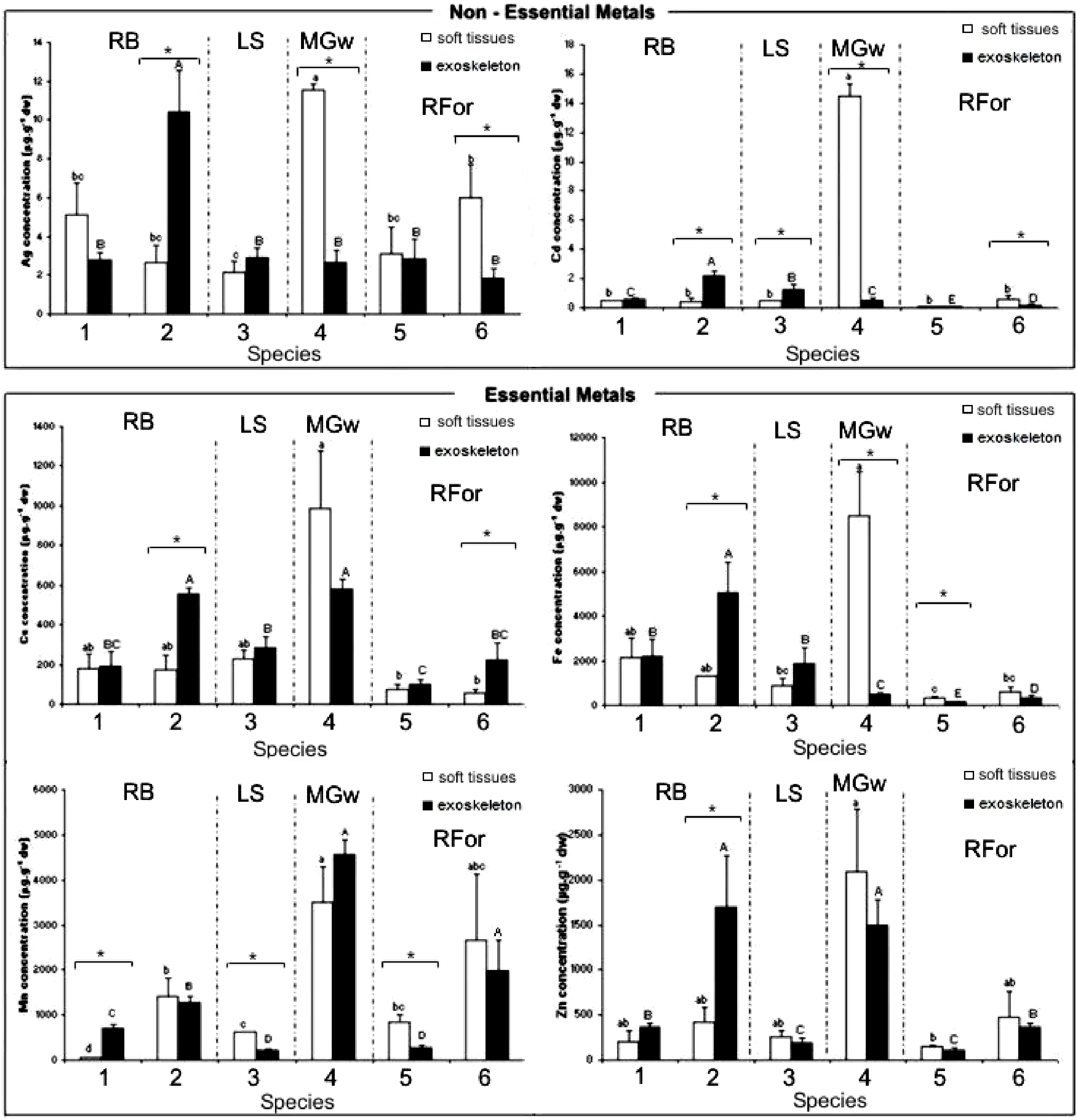

Figure 1: Non-essential (Ag and Cd) and essential metal concentrations (Cu, Fe, Mn and Zn) (µg. g^{-1} dry weight) in soft and exoskeleton tissues of shrimp species. *R. exoculata* (1)*, M. fortunata* (2) and *C. chacei* (3,4) from Mid- Atlantic hydrothermal vent fields *and P. elegans* (5) and *P. varians* (6) from Ria Formosa coastal area (Portugal). The data represent average and ± 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 ($\rho > 0.05$). * Significant differences between soft tissues and exoskeleton for each species within every metal ($\rho < 0.05$).

Table I: 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 ± standard deviation (SD) for n= 24. Values followed by the same letter are not statistically different ($\rho > 0.05$)

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

* Adapted from Gonzalez-Rey et al., 2007

n.d. not determined

Chapter 3

Can antioxidant enzymes of vents and coastal shrimp species also protect against metal-toxicity?

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ORIGINAL ARTICLE

Adaptation to metal toxicity: a comparison of hydrothermal vent and coastal shrimps

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Keywords
Antioxidant enzymes; hydrothermal vents; metallothionein; *Mirocaris fortunata*; *Palaemon elegans*; *Palaemonetes varians*; Ria Formosa; *Rimicaris exoculata*; shrimps.

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Abstract

Rainbow vent field is one of the most metal-contaminated hydrothermal sites on the Mid-Atlantic Ridge near the Azores region. Two hydrothermal shrimp species dominate the fauna at the Rainbow site along with the mussel *Bathymodiolus azoricus*. Although the levels of essential and non-essential metals in these shrimps have been studied, the biological consequences of a metal-rich environment are still largely unknown. Therefore, the aim of this study was to determine the levels of metal-binding proteins – metallothioneins (MT) and the activities of antioxidant enzymes – superoxide dismutase, catalase, total glutathione peroxidase and selenium-dependent glutathione peroxidase in two hydrothermal vent shrimps (*Mirocaris fortunata* and *Rimicaris exoculata*) collected from the Rainbow site and to compare them with two coastal shrimps (*Palaemon elegans* and *Palaemonetes varians*) from a south Portugal lagoon (Ria Formosa) to evaluate their different adaptation strategies towards metals in their environment. Results show significant differences in MT levels and antioxidant enzymatic activities between vent and coastal shrimps and also between shrimp species collected from the same site. This suggests that biochemical responses in both vent and coastal shrimps are affected not only by the environmental characteristics but also by inter-specific differences. Nevertheless, these responses apparently confer successful adaptation for survival in a metal-extreme environment.

Problem

Hydrothermal vent environments exhibit natural high metal concentrations as a result of the interaction between seawater and magmatic rocks. Consequently, the hydrothermal vent fluids have a very high temperature (300–350 °C) and are naturally enriched in silica (SiO_2), metals (*e.g.* Fe, Mn, As, Cd, Cu, *etc.*) and dissolved gases (*e.g.* H_2S, CH_4, H_2, CO_2) (Von Damm 1990, 1992; Lowell *et al.* 1995; Von Damm *et al.* 1995; Sarradin *et al.* 1998).

The Rainbow vent field was discovered in 1997, is located at 36° 13.8′ N 33° 54.15′ W in the north AMAR (ALVIN Mid-Atlantic Ridge) segment, and is the deepest vent site located in the Azores Triple Junction area (2270–2500, 2320 m deep) (Fouquet *et al.* 1997; Desbruyères *et al.* 2001; Douville *et al.* 2002). The most active smokers are located at the western and the eastern ends of the hydrothermal field (Desbruyères *et al.* 2001). Rainbow hydrothermal fluids are uniform in composition and influenced by phase separation (Douville *et al.* 1999). Active chimneys, emitting hot-temperature (365 °C) acidic fluids ($pH = 2.8$) and that are relatively low in H_2S, are arranged longitudinally along a 200-m stretch of ridge. The metal concentrations (Cu, Fe, Mn, and Zn) in Rainbow vent fluids are the highest observed in the MAR hydrothermal area (Desbruyères *et al.* 2000; Douville *et al.* 2002).

In contrast, the Ria Formosa is a highly productive mesotidal lagoon, separated from the ocean by a system of five sand barrier islands and six inlets, which extend for about 55 km along the south coast of Portugal.

The average water depth is <2 m and the tidal height varies from a maximum of 3.7 m at spring tide to a minimum of 0.4 m at neap tide. Hence, most of the water volume is drained in each tidal cycle, thereby imposing a short residence time and an intense exchange of materials between the Ria Formosa and the adjacent coastal waters (Bebianno 1995; Caetano *et al.* 2002; Santos *et al.* 2004). Nevertheless, the water quality of the lagoon has deteriorated over the last decade reflecting the intense economic development of areas around the lagoon, whose major inputs of pollutants came from untreated sewage and domestic effluents from two cities (Bebianno 1995; Caetano *et al.* 2002).

Caridean shrimps, in particular *Mirocaris fortunata* (Martin & Christiansen 1995) and *Rimicaris exoculata* (William & Rona 1986), co-dominate populations in deep Atlantic hydrothermal fields near the Azores Triple Junction. The shrimp species *M. fortunata* colonize mainly sulphide diffusers, with higher densities on chimneys covered by iron oxides. In contrast, dense swarms of *R. exoculata* are located in small depressions between chimneys expelling superheated sulphide-loaded fluid (Gebruk *et al.* 1993, 2000; Segonzac *et al.* 1993; Polz *et al.* 1998; Desbruyères *et al.* 2001). These species have been extensively studied in terms of their abundance, density and microhabitat preferences in MAR hydrothermal vents (Gebruk *et al.* 1993, 2000; Segonzac *et al.* 1993; Desbruyères & Segonzac 1997; Polz *et al.* 1998), behaviour and nutritional strategies (Casanova *et al.* 1993; Renninger *et al.* 1995; Gebruk *et al.* 2000) and concentration of essential and non-essential metals in their tissues (Geret *et al.* 2002; Kádár *et al.* 2006). Nevertheless, the physiological responses towards metals in these species are still largely unknown. It is well recognized that metals can be toxic to organisms when present at high levels. Metals can increase the synthesis of metallothioneins (MT), which bind free metal ions (Langston *et al.* 1998) to form an inactive metal–MT complex and therefore these proteins are capable of detoxifying the metals inside the cells (Park *et al.* 2001). The accumulation of metals also enhances the production of highly toxic radical oxygen species (ROS) (Fridovich 1998), which include the superoxide anion radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical ($OH^\bullet$), peroxyl radicals ($ROO^\bullet$), alkoxyl radicals ($RO^\bullet$) and peroxynitrite (HO-ONO) (Darley-Usmar *et al.* 1995). Hydrogen sulphide, known to be a potent inhibitor of antioxidant enzymes, reacts spontaneously with oxygen to generate many toxic oxygen and sulphide compounds, which in turn are capable of inflicting DNA damage (Pruski & Dixon 2003). Aerobic organisms possess a baseline status of antioxidant systems, involved in a variety of detoxification reactions, to assure the maintenance of a balance between production and removal of endogenous ROS and other pro-oxidants. This pro-oxidant/antioxidant balance and detoxification of potentially damaging ROS is crucial for cellular homeostasis (Winston & Di Giulio 1991; Lemaire & Livingstone 1993; Livingstone 2001). The activities of antioxidant enzymes were described for some hydrothermal vent species, mainly in the mussels *Bathymodiolus azoricus* from MAR vent fields (Bebianno *et al.* 2005), in the tubeworm *Riftia pachyptila* and in the clam *Calyptogena magnifica* from the East Pacific Rise vent fields (Blum & Fridovich 1984).

Nevertheless, there is still a lack of information about the antioxidant defence systems in other hydrothermal species and their relation with environmental characteristics. Therefore, the aim of this study was to address several questions regarding the metal detoxification processes in both hydrothermal and costal shrimps, namely:

1 Are MT concentrations more similar in shrimps from the same environments? Is there any relationship between MT levels and antioxidant enzyme activities?

2 Is there a relation between the metabolic responses towards metals in shrimps from different environments?

3 Can these responses be due to specific environmental parameters related to microhabitat, or to inter-specific characteristics like feeding strategies?

To answer these questions we studied the adaptation strategies towards metals, especially those regarding MT levels and activities of antioxidant enzymes in two hydrothermal vent shrimp species (*M. fortunata* and *R. exoculata*) from the Rainbow vent field. We compared the results with two euryhaline coastal shrimp species (*Palaemon elegans*, Rathke 1837 and *Palaemonetes varians*, Leach 1814), with analogous microhabitat and feeding habits, *i.e.* detritivores (FAO – Food and Agriculture Organization of the United Nations 1980) collected in a lagoon system (Ria Formosa).

Material and Methods

Two hydrothermal vent shrimp species, *Rimicaris exoculata* (56.89 ± 7.32 mm carapace length, n = 8) and *Mirocaris fortunata* (21.89 ± 2.06 mm carapace length, n = 8) were collected from the Rainbow vent site, located on the Mid-Atlantic Ridge (MAR) (36°13′ N; 33°54.1′ W, 2500 m) (Fig. 1A), using the remote operated vehicle VICTOR6000 during the SEAHMA I cruise. Additionally, two coastal shrimp species, *Palaemon elegans* (36.29 ± 4.18 mm carapace length, n = 8) and *Palaemonetes varians* (33.47 ± 2.21 mm carapace length, n = 8), were collected from the margin of the Ria Formosa lagoon system in south Portugal (37°03′ N; 07°47′ W) (Fig. 1B) with a sub-superficial tow using a shrimp net (*c.* 40 cm diameter). The two coastal shrimp species were sampled at the same local site. The physical–chemical

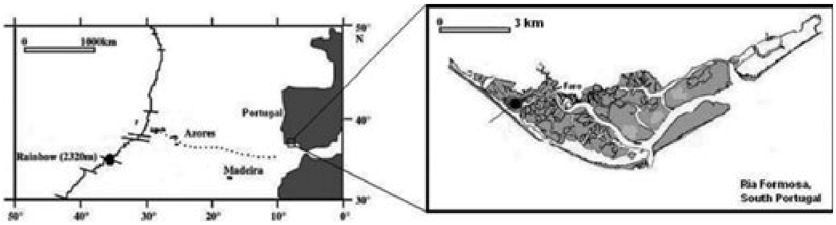

Fig. 1. Location of Rainbow hydrothermal vent site in the Azores Triple Junction area (A) and Ramalhete channel in the Ria Formosa lagoon (South Portugal) (B) (adapted from Caetano *et al.* 2002; Santos *et al.* 2004; Bebianno *et al.* 2005).

comparison between the two sampling sites is presented in Table 1. Both vent and coastal shrimp species were immediately frozen in liquid nitrogen after collection and stored at −80 °C until biochemical analysis. All dissected shrimp exoskeletons and gills were separated from soft tissues.

Metallothioneins

To determine the MT concentrations, the whole soft tissues of hydrothermal and coastal shrimps were homogenized in three volumes of 0.02 м TRIS-HCl buffer (pH 8.6) in an ice bath (4 °C). An aliquot of the homogenate (3 ml) was centrifuged at 30,000 *g* for 1 h at 4 °C. The supernatant (cytosol) was separated from the residual fraction, heat-treated at 80 °C for 10 min to precipitate the high molecular weight ligands, and subsequently centrifuged under the conditions described above. Aliquots of the heat-treated cytosol were used for the quantification of MT concentrations by differential pulse polarography according to the method described by Bebianno & Langston (1989). MT levels are expressed as mg·g^{-1} total protein concentrations.

Table 1. Temperature, pH and concentration of chemical species in the end-member fluids of lagoon system Ria Formosa (South Portugal) and MAR vent field (Rainbow) compared with average seawater (adapted from Caetano *et al.* 1997; Douville *et al.* 2002).

Site	Ria Formosa 37°03′ N; 07°47′ W	Rainbow 36°13′ N, 33°54′ W	Seawater
T (°C)	17.3[a]	365[c]	–
pH	8.28[a]	2.8[c]	7.8
H_2S (mм)	–	1.0[c]	~0
CO_2(mм)	–	< 16[c]	–
CH_4(mм)	–	2.2–2.5[c]	~0
Cd (nм)	0.9 – 4.5[a]	130[c]	0.7
Cu (μм)	0.02 – 0.05[a]	140[c]	0.0033
Zn (μм)	0.02 – 0.03[a]	160[c]	0.028
Fe (μм)	8 – 52[b]	24000[c]	0.0045
Mn (μм)	2.5 – 6.3[b]	2250[c]	0.0013
Cl (mм)	–	750[c]	546
Co (μм)	–	13[c]	<2
Ag (nм)	–	47[c]	0.023
Ni (μм)	–	3[c]	<2
Si (mм)	–	6.9[c]	<0.2

[a] Instituto Hidrográfico (1998).
[b] Caetano *et al.* (1997).
[c] Douville *et al.* (2002).

Antioxidant enzymes

Antioxidant enzymatic activities were determined in the different shrimp species' edible tissues, after homogenization in 20 mм Tris buffer, pH 7.6, containing 1 mм of EDTA, 0.5 м of saccharose, 0.15 м of KCl and 1 mм of DTT. The homogenates were centrifuged at 500 *g* for 15 min at 4 °C to precipitate large particles and centrifuged again at 12,000 *g* for 45 min at 4 °C to precipitate the mitochondrial fraction. Supernatants were purified on a Sephadex G-25 gel column to remove low molecular weight proteins.

Superoxide dismutase (SOD) activity (EC 1.15.1.1) was determined by measuring the reduction of cytochrome *c* by the xanthine oxidase/hypoxanthine system at 550 nm (McCord & Fridovich 1969). One unit of SOD is defined as the amount of enzyme that inhibits the reduction of cytochrome *c* by 50%. SOD activity is expressed in U SOD mg^{-1} total protein concentrations.

Catalase (CAT) activity (EC 1.11.1.6) was determined according to Greenwald (1985) by the decrease in absorbance at 240 nm because of H_2O_2 consumption. The CAT activity is expressed as mmoles min^{-1} mg^{-1} of total protein concentrations.

Glutathione peroxidase activities were measured following NADPH oxidation at 340 nm in the presence of excess glutathione reductase, reduced glutathione and corresponding peroxide (Lawrence & Burk 1976). The selenium-dependent glutathione peroxidase (Se-GPx) (EC 1.11.1.9) and total glutathione peroxidase (GPx) activities were measured by using respectively, H_2O_2 and cumene hydroperoxide as substrates. GPx activities are expressed as μmoles min^{-1} mg^{-1} of total protein concentrations.

Total protein concentrations

The whole edible tissues of vent and coastal shrimp were homogenized in 20 mм Tris buffer, pH 8.6, containing

150 mM of NaCl. The homogenates were centrifuged for 30 min at 30,000 *g* at 4 °C. Total protein concentrations were measured on supernatants by the Lowry method (Lowry *et al.* 1951) using BSA as reference standard material. Protein concentrations are expressed as mg·g^{-1} wet weight tissue.

The variability of MT concentrations and antioxidant enzymes activities was tested in the different species through the analysis of variance (one way-ANOVA). A Duncan test was used to determine significant differences between species for each variable. Regression analyses were also applied to assess the relationship between the concentrations of MT and antioxidant enzymes. A significance level of 0.05 was used for all statistical analysis, *i.e.* probability of $P \le 0.05$ was considered significant.

Results

In the hydrothermal vent shrimp from Rainbow, MT levels in *Rimicaris exoculata* (7.30 ± 0.66 mg·g^{-1} ww protein) were approximately sixfold higher compared with those found in *Mirocaris fortunata* (1.27 ± 0.27 mg·g^{-1} ww protein) ($p < 0.05$) (Fig. 2), whereas MT concentrations in *Palaemon elegans* were approximately 2.5-fold higher than in *Palaemonetes varians* ($P < 0.05$) (4.34 ± 0.99 mg·g^{-1} ww protein and 1.65 ± 0.39 mg·g^{-1} ww protein, respectively) (Fig. 2) and were not significantly different from *M. fortunata*. These results do not support the theory that MT concentrations are directly related to the environment where the shrimps were collected. Thus, MT concentrations followed the order: *R. exoculata* > *P. elegans* > *M. fortunata* = *P. varians*. Nevertheless, the higher MT levels in *R. exoculata* suggest that this shrimp species is more exposed to metal-contamination.

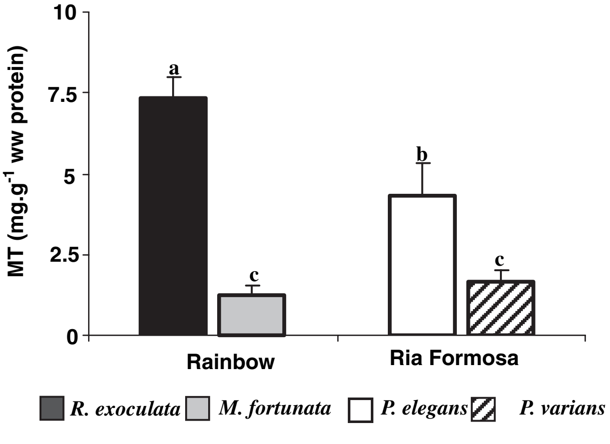

Fig. 2. Metallothionein concentrations (MT) in the edible tissues of hydrothermal (*Rimicaris exoculata* and *Mirocaris fortunata*) and coastal shrimp (*Palaemon elegans* and *Palaemonetes varians*). The data represent average ± standard deviation (SD), n = 16. Values followed by the same letter are not statistically different ($p > 0.05$).

Regarding antioxidant enzymes activities, all the results obtained, except the ones for cytosolic CAT activity, show a negative relationship with MT levels, ($MT = -0.345\ SOD + 6.2$, $r = 0.728$, $P < 0.05$; $MT = -175.0$ total $GPx + 7.5$, $r = 0.858$, $P < 0.05$; $MT = -1344.8\ Se\text{-}GPx + 11.8$, $r = 0.911$, $P < 0.05$). The cytosolic SOD activity was significantly different among all sampled shrimp species (ANOVA, $F_{3,30} = 55.89$, $P < 0.001$). Cytosolic SOD activity was significantly higher in *M. fortunata* from Rainbow (1615.99 ± 5.66 U mg^{-1} protein) compared with the other vent and coastal species ($p < 0.05$). At the same time, the hydrothermal vent shrimp *R. exoculata* exhibited the lowest SOD activity (2.56 ± 0.66 U mg^{-1} protein) (Fig. 3A). No significant differences in the activity of cytosolic SOD were found between the two coastal shrimp species, *P. elegans* and *P. varians*, from the Ria Formosa Lagoon ($P > 0.05$) (5.14 ± 1.58 and 5.67 ± 1.73 U mg^{-1} protein, respectively) (Fig. 3A).

All shrimp species had significantly different cytosolic CAT activity (ANOVA, $F_{3,26} = 28.60$, $P < 0.001$). The activity of cytosolic CAT was approximately threefold higher in the two vent shrimp species (0.0042 ± 0.0005 mmoles min^{-1} mg^{-1} protein for *R. exoculata* and 0.0048 ± 0.001 mmoles min^{-1} mg^{-1} protein for *M. fortunata*) compared with their coastal counterparts (0.0014 ± 0.0005 mmoles min^{-1} mg^{-1} protein for *P. elegans* and 0.002 ± 0.0005 mmoles min^{-1} mg^{-1} protein for *P. varians*) ($P > 0.05$) (Fig. 3B). Each and every analysed shrimp species was significantly different for total GPx activity (ANOVA, $F_{3,26} = 18.47$, $P < 0.001$). The antioxidant enzyme levels followed a similar pattern of cytosolic SOD, where a significantly higher activity was observed in *M. fortunata* (0.040 ± 0.01 μmoles min^{-1} mg^{-1} protein) compared with all the other shrimp species ($p < 0.05$) (Fig. 3C). As occurred for SOD and CAT activities, no significant differences were observed in total GPx between coastal shrimp *P. elegans* (0.023 ± 0.004 μmoles min^{-1} mg^{-1} protein) and *P. varians* (0.015 ± 0.007 μmoles min^{-1} mg^{-1} protein) collected in the Ria Formosa Lagoon ($p > 0.05$). Moreover, the hydrothermal vent shrimp *R. exoculata* exhibited no significant differences compared with *P. varians* ($P > 0.05$) (Fig. 3C). As occurred for all above antioxidant enzymes, Se-GPx activity was significantly different for all shrimp species (ANOVA, $F_{3,27} = 4.32$, $P < 0.01$). This enzyme activity represents approximately one third of the total GPx activity in both hydrothermal and coastal shrimp species (Fig. 3D). No significant differences were found in GPx activities between the hydrothermal shrimp *M. fortunata*

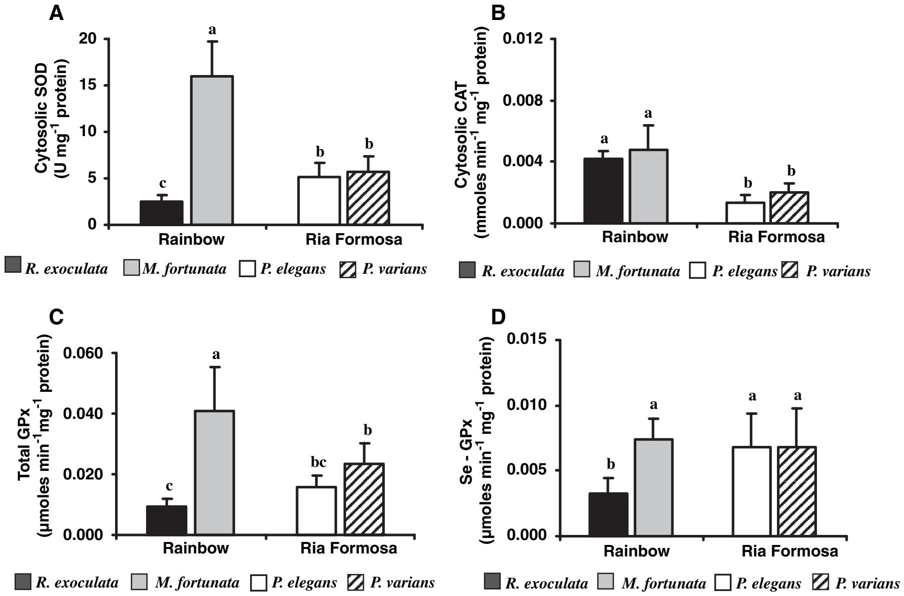

Fig. 3. Antioxidant enzymes activities of (A) cytosolic superoxide dismutase; (B) cytosolic catalase; (C) Total glutathione peroxidase and (D) selenium-dependent glutathione peroxidase in edible tissues of hydrothermal (*Rimicaris exoculata* and *Mirocaris fortunata*) and coastal shrimp (*Palaemon elegans* and *Palaemonetes varians*). The data represent average and ± standard deviation (SD) for n = 16. Values followed by the same letter are not statistically different ($p > 0.05$).

(0.007 ± 0.001 μmoles min^{-1} mg^{-1} protein) and both coastal species from Ria Formosa ($p > 0.05$). On the other hand, Se-GPx activity in *R. exoculata* (0.003 ± 0.001 min^{-1} mg^{-1} protein) was approximately half of that observed for the other vent and coastal species ($p < 0.05$) (Fig. 3D).

As occurred for MT levels, no direct relationships were found in antioxidant enzyme activities between the two shrimp species from Rainbow site; however, their coastal counterparts do not show significant differences between them. Direct correlation with environment was only found in cytosolic CAT activity, but there were no significant differences between shrimp species within hydrothermal vent and coastal environments.

Discussion

An intriguing vent paradox is how to reconcile the fast growth rates and abundant biomass that typify vent species with the highly toxic and stressful nature of their deep-sea environment (Dixon *et al.* 2000). This is particularly evident at the Rainbow hydrothermal vent site, where the highest metal concentrations in MAR hydrothermal area can be found (Douville *et al.* 1997, 2002; Desbruyères *et al.* 2000) and caridean shrimp species *Rimicaris exoculata* and *Mirocaris fortunata* together with the vent mussel *Bathymodiolus azoricus* co-dominate the Rainbow hydrothermal vent megafauna.

Metallothioneins have been proposed as a biomarker of metallic exposure in several organisms (Amiard & Cosson 1997). Amiard *et al.* (2006) recently compiled several studies showing a clear correlation between metallic concentrations with MT levels, and an evident induction of this protein after Cd, Cu and Zn exposures in aquatic invertebrates, including decapod shrimps. Crustacean species are widely used as biological indicators of environmental alterations, as they play a key ecological role as planktivorous grazers, epibenthic scavengers or as prey species (Clark 1989). Generally, these studies are focussed mainly on the hepatopancreas tissue as it has a central role in the metabolism, storage and detoxification of metals (Pourang *et al.* 2004). In the present work, however, we considered the whole soft tissue due to technical constraints for antioxidant enzyme determination.

As antioxidant enzymes can also protect against metal-induced reactive oxygen species, it is important to understand both MT and antioxidant enzymatic responses in these organisms. Therefore, this study was the first attempt to compare MT levels and antioxidant defence systems in four shrimp species from hydrothermal vent and coastal environments and try to explain the importance of such responses in the resistance and tolerance of these species to a metal-rich environment.

Considering the possible influence of the environment in MT concentrations, the results obtained showed that MT levels were markedly different between shrimp species from each vent and coastal site. We expected a higher similarity in MT concentrations between shrimp from the same type of environment, as the Rainbow shrimp exhibited higher metal concentration levels in their fluids and in whole body tissue burden (Kádár *et al.* 2006 obtained for *Rimicaris*: 35.6×10^3 μg·g^{-1} dry weight Fe, 1.8×10^3 μg·g^{-1} dry weight Zn, 0.8×10^3 μg·g^{-1} dry weight Cu and for *Mirocaris*: 6.6×10^3 μg·g^{-1} dry weight Fe, 2.5×10^3 μg·g^{-1} dry weight Zn, 1.0×10^3 μg·g^{-1} dry weight Cu) when compared with the coastal species collected in the Ria Formosa Lagoon (data not shown). However, this was only true for *R. exoculata*, while the other vent shrimp, *M. fortunata*, had MT concentrations similar to those found for the coastal species *Palaemon elegans*. Although *M. fortunata* are found more distant from active venting than *R. exoculata*, and therefore have a lower exposure to the vent fluids, they seem to accumulate more metals in their tissues, which may suggest more efficient detoxification strategies to this potentially toxic environment (Kádár *et al.* 2006).

Therefore, the differences observed in MT levels between vent and coastal shrimp can derive from interspecific differences in the basal levels of these proteins, rather than reflecting a metabolic response to their environments. Geret *et al.* 2002 found similar MT levels in *R. exoculata* from Rainbow hydrothermal field. However, no information concerning the basal MT levels in *M. fortunata*, *P. elegans* and *Palaemonetes varians* is available. Also, in crustaceans a relatively high amount of metals is associated with the insoluble forms (Geret *et al.* 2002) that can be mobilized and immobilized during the moult cycle (Engel & Brouwer 1991, 1993). In hydrothermal vent shrimp, intra-specific adaptations to deep-sea hydrothermal conditions can also derive from their different nutritional behaviours, *i.e. M. fortunata* has been described as an opportunistic scavenger while *R. exoculata* possess symbiotic bacteria in their branchial chambers (Gebruk *et al.* 2000). Nevertheless, we can also hypothesize that, although coastal shrimp exhibit the same sampling environment and analogous feeding habits, vent shrimp have different microhabitats (described earlier). Unfortunately no available data concerning metal concentration levels in the surrounding water of each microhabitat were found in the literature to confirm this assumption.

In general, the antioxidant enzyme activities in the two hydrothermal shrimp species are significantly lower than those found for the mussel *B. azoricus* from the same hydrothermal vent site, except the cytosolic SOD activity (5.52 ± 1.08 U·mg^{-1} protein), which was the same order of magnitude (Bebianno *et al.* 2005). Hydrothermal vent mussel species *B. azoricus* at Rainbow site exhibit much higher cytosolic CAT, total GPx and Se-GPx activities. However, cytosolic SOD activity (5.52 ± 1.08 U·mg^{-1} protein) seems to be in agreement with that obtained in shrimp species, although being closer to coastal shrimp species rather than hydrothermal vent shrimp species.

Concerning the relationship between MT levels and antioxidant enzymes, results point out that in general, antioxidant enzyme activities (especially cytosolic SOD, Total GPx and Se-GPx) in both vent and coastal shrimp species had an inverse pattern compared with the MT levels, suggesting a negative relationship between these two protection systems. However, this negative relationship is more noticeable among vent shrimp species. Although antioxidant enzymes are the main factor responsible for ROS detoxification inside the cells, the antioxidant properties of MT have also been described, mainly in the elimination of the hydroxyl radical (Chubatsu & Meneghini 1993). This suggests that when MT levels in vent and coastal shrimp are enhanced, they are more efficiently sequestered from the intracellular medium. Consequently, metal-induced reactive oxygen species are less likely to be formed when MT synthesis increases, leading to a natural decrease in the antioxidant enzymatic protections.

Even so, the biochemical responses towards metals in coastal shrimp are more similar between them, than between hydrothermal vent shrimp. Thus, microhabitat and feeding habitats are the crucial variables for metal uptake and consequently will influence the metal detoxification systems.

Summary

Very few studies deal with the specific biochemical responses from hydrothermal vent organisms as an adaptation to their extreme environment. Shrimp are key species in these environments as they dominate the hydrothermal vent fauna along with hydrothermal vent mussels of the genus *Bathymodiolus*. Results obtained suggest that biochemical responses in vent and coastal shrimp are not only affected by environmental characteristics but also by interspecific differences. The detoxification strategies towards metals (MT and antioxidant enzymes) observed in several shrimp species suggest distinct metabolic responses; nevertheless these responses confer successful adaptations to survival in a metal-extreme environment.

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Chapter 4.

1. General Discussion and Conclusions

Hydrothermal ecosystems of the Mid Atlantic Ridge (MAR) are dominated by shrimp and mussels that are naturally exposed to elevated levels of metals providing unique in situ laboratories for ecotoxicological investigations (Kádár *et al.*, 2006).

The present study show that in all shrimp species the most accumulated metals are Fe, Zn, Mn and Cu while Ag and Cd are the less accumulated. The accumulation of metals is mainly due to their essentiality and content in the surrounding water where shrimp dwell.

In hydrothermal vent species, the accumulation pattern reflects the metallic concentration in the hydrothermal fluids, confirming extreme exposure levels at the geochemically different hydrothermal vents. The concentration of metals present in different tissues of crustaceans vary widely between metals and between taxa (Rainbow, 1998). As described above for most hydrothermal shrimp species, Fe is the prominent metal, whereas for coastal counterparts is Mn.

Regarding metal concentration in soft tissues, vent shrimp species present the subsequent order, for *R. exoculata*: Fe > Mn > Zn > Cu > Ag > Cd; for *M. fortunata*: Fe > Mn > Zn > Cu > Ag > Cd; both *C. chacei* (from Lucky Strike and Menez-Gwen) exhibit exactly the same accumulation pattern as *R. exoculata*. Concerning coastal shrimp species, the metals concentration order is equivalent: Mn > Fe > Zn > Cu > Ag > Cd.

Concerning metal concentration in exoskeleton, vent shrimp species exhibit the following pattern for *C. chacei* (from Lucky Strike): Fe > Cu > Mn > Zn > Ag > Cd; for *C. chacei* (from Menez-Gwen): Mn > Zn > Cu > Fe > Ag > Cd; both *R. exoculata* and *M. fortunata* display exactly the same sequence: Fe > Mn > Zn > Cu > Ag > Cd (Table III).

The fact that both *M. fortunata* from Rainbow and *C. chacei* from Lucky Strike denote significantly higher accumulation of metals in the exoskeleton compared to soft tissues, and coastal shrimp present the opposite, may be an example that the overwhelmed metallic concentration found in vent fields favours moulting as an additional mechanism of detoxification.

Like metal levels, results show significant differences in metallothionein levels between vent and coastal shrimps. MT levels present the following order: *C. chacei* (LS) > *R. exoculata* > *P. elegans* > *C. chacei* (MGw) ≥ *P. varians* = *M. fortunata.*

The differences observed in MT levels can derive from interspecific differences in the basal levels of these proteins, rather than reflecting a metabolic response to their environments. Nevertheless, positive correlation exist between MT levels and some metals, namely with Ag (in *P. elegans*), with Zn (in *P. varians*) and with Cd (in *C. chacei* from Lucky Strike) indicating that MT may have a major role in the detoxification of these metals in these shrimp species.

In general, the obtained antioxidant enzyme activities in the two hydrothermal shrimp species (from Rainbow) are significantly lower than those found for the mussel *B. azoricus* from the same hydrothermal vent site, except for cytosolic SOD activity, which is in the same order of magnitude (Bebianno *et al*., 2005).

Unlike metallothionein levels, no correlation was detected between antioxidant enzymes activities and metals concentrations.

Although *C. chacei* from Lucky Strike displays the highest MT levels, which are four-fold higher than the ones obtained for the same species from Menez-Gwen vent field, LPO levels were significantly lower than those of *R. exoculata* and *M. fortunata* from RB and similar to the coastal shrimp. Thus, MT (particularly for *C. chacei* from LS) may have an antioxidant role in reactive oxygen species detoxification inside the cells, mainly in the elimination of hydroxyl radical, as described by Chubatsu & Meneghin (1993).

As antioxidant enzymes also protect against metal induced ROS, it is important to understand both MT and antioxidant enzymatic responses in these organisms. Therefore, results point out that antioxidant enzyme activities (especially cytosolic SOD, Total GPx and Se-GPx) in both vent and coastal shrimp species show an inverse pattern compared with the MT levels, suggesting a negative relationship (which is more noticeable among vent shrimp species) between these two protection systems.

Additionally, the higher LPO levels in Rainbow hydrothermal vent field species point out to an elevated shrimp exposure to stressful environmental conditions, which is in agreement to the fact that Rainbow vent is the site with higher metals concentrations in MAR. This is particularly evident when considering the case between *M. fortunata* and *P. varians* species, where no significant differences in MT levels were found and *M. fortunata* presented four-fold higher LPO levels.

Finally, results suggest that biochemical responses in both vent and coastal shrimp are not only affected by environmental characteristics (such as microhabitat) but also by interspecific differences (such as feeding habits and moulting cycles), nevertheless, these responses apparently grant a successful adaptation to survival in metal-rich environments.

Table III. Essential (Cu, Fe, Mn and Zn) and non-essential metal concentrations (Ag and Cd) (µg/g dry weight) in both soft and exoskeleton tissues of shrimp species: *R. exoculata*, *M. fortunata*, *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 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 (ρ>0.05).

Site	Species	Essential Metals								Non-Essential Metals			
		Cu µg/g dw (standard deviation)		Fe µg/g dw (standard deviation)		Mn µg/g dw (standard deviation)		Zn µg/g dw (standard deviation)		Ag µg/g dw (standard deviation)		Cd µg/g dw (standard deviation)	
		Soft tissues	Exoskeleton	Soft tissues	Exoskeleton	Soft tissues	Exoskeleton	Soft tissues	Exoskeleton	Soft tissues	Exoskeleton	Soft tissues	Exoskeleton
Rainbow	*Rimicaris exoculata*	176.76^{ab} (74.09)	190.73^{BC} (71.78)	2122.41^{ab} (867.85)	2197.76^{B} (743.91)	43.24^{d} (10.45)	701.99^{C} (91.58)	209.27^{ab} (111.53)	363.53^{B} (50.41)	5.08^{bc} (1.68)	2.77^{B} (0.39)	0.49^{b} (0.02)	0.58^{C} (0.06)
	Mirocaris fortunata	172.86^{ab} (75.22)	556.70^{A} (27.09)	1302.49^{ab} (16.77)	5060.49^{A} (1349.37)	1409.88^{b} (396.28)	1274.56^{B} (147.62)	418.38^{ab} (169.70)	1702.47^{A} (558.78)	2.68^{bc} (0.88)	10.40^{A} (2.16)	0.46^{b} (0.19)	2.17^{A} (0.37)
Lucky Strike	*Chorocaris chacei*	228.00^{ab} (44.78)	286.40^{B} (54.02)	876.78^{bc} (333.33)	1875.31^{B} (697.11)	614.92^{c} (17.63)	222.85^{D} (19.59)	251.36^{ab} (79.68)	191.35^{C} (57.86)	2.12^{c} (0.59)	2.89^{B} (0.53)	0.49^{b} (0.01)	1.30^{B} (0.29)
Menez-Gwen	*Chorocaris chacei*	988.25^{a} (291.46)	581.77^{A} (44.26)	8508.00^{a} (1938.00)	510.83^{C} (58.62)	3494.45^{a} (802.26)	4568.92^{A} (313.18)	2089.00^{a} (689.00)	1488.88^{A} (287.70)	11.53^{a} (0.33)	2.65^{B} (0.60)	14.50^{a} (0.85)	0.54^{C} (0.13)
Ria Formosa	*Palaemons elegans*	73.99^{b} (23.37)	99.52^{C} (21.97)	328.29^{c} (50.40)	138.38^{E} (43.08)	836.65^{bc} (161.14)	279.10^{D} (44.25)	151.39^{b} (7.12)	102.51^{C} (34.95)	3.10^{bc} (1.40)	2.81^{B} (1.02)	0.07^{b} (0.01)	0.07^{E} (0.02)
	Palaemonetes varians	54.92^{b} (21.10)	221.24^{BC} (84.65)	594.94^{bc} (246.70)	320.51^{D} (97.48)	2661.69^{abc} (1478.31)	1976.81^{B} (679.52)	470.21^{ab} (294.40)	367.97^{B} (42.43)	6.02^{b} (1.70)	1.83^{B} (0.48)	0.61^{b} (0.20)	0.17^{D} (0.05)

2. Future Perspectives

In order to better understand metal detoxification mechanisms in shrimp, it would be pertinent to proceed to:

- An increase of sample numbers, reducing the constraints due to minimal weight biochemical analysis, and additionally accredit the results obtained to the shrimp populations,
- A more detailed characterization of both hydrothermal vent and coastal shrimp species microhabitat, namely performing further physical-chemical analysis of surrounding sediment and water,
- Additional studies about vent species moulting cycles and feeding behaviours, expressly in the comprehension of the symbiosis relationship with bacteria.
- Further investigations about shrimp metabolism toward metals, namely the recognition of how each metal is accumulated and which are their specific functions,
- The identification of specific metal/metallothionein correlations in shrimp and perform MT induction studies in coastal species,
- The analysis and identification of the metallic ions present in insoluble granules in shrimp species,
- A proteomic analysis of the coastal and vent shrimp species; their comparison could radically enhance the knowledge about hydrothermal vent species metabolic adaptation to metal-rich environments.

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