Cah. Biol. Mar. (2002) 43 : 43-52

Metal bioaccumulation and storage forms in the shrimp, *Rimicaris exoculata*, from the Rainbow hydrothermal field (Mid-Atlantic Ridge); preliminary approach to the fluid-organism relationship

Florence GERET[1], Ricardo RISO[1], Pierre-Marie SARRADIN[3], Jean-Claude CAPRAIS[3] and Richard P. COSSON[1*]

[1] *ISOMer, Laboratoire de Biologie Marine - BP 92 208, F-44 322 - Nantes Cedex 3, France*

[2] *Laboratoire de Chimie Marine, IUEM / UBO, UPR 9042, CNRS - F-29280 Plouzané, France*

[3] *IFREMER, Centre de Brest, DRO/EP, BP 70, F-29280 Plouzané, France*

**correspondence to :*

Richard P. Cosson. Fax : (33) 2 51 12 56 68; E-mail : cosson@isomer.univ-nantes.fr

Abstract: At the Rainbow hydrothermal site the concentrations of metals in the fluid are the highest observed in the Mid-Atlantic Ridge hydrothermal areas, and the shrimps *Rimicaris exoculata* were observed forming dense swarms in small depressions on the chimney walls. In order to understand the fluid-organism relationship, we investigated the relative abundance of metals in the water surrounding *R. exoculata* and their bioaccumulation by the shrimps. This first approach stresses a net metal enrichment around *R. exoculata*. Precipitation, scavenging and oxidative redissolution processes in the mixing zone are controlling the concentrations of metals. Their bioaccumulation varies according to their bioavailability in the surrounding water. Metals are abundant as insoluble forms in the tissues but the hypothesis of external deposits on the gills and the cuticle of the abdomen cannot be rejected. The regular lysosomal breakdown of metallothioneins may participate to the relative abundance of these insoluble forms. Improvements concerning the preparation of the biological material and the use of a complementary histological technique are needed to answer the questions emerging from our work. The study of the distribution of metals between dissolved and particulate fractions in the water surrounding the shrimps has to be performed, in order to provide data on the bioavailability of metals.

Résumé: *Bioaccumulation des métaux et formes de stockage chez la crevette* Rimicaris exoculata *(Ride Médio-Atlantique) ; approche préliminaire de la relation entre le fluide et l'organisme.* Sur la ride Médio-Atlantique, les plus fortes concentrations en métaux dans le fluide hydrothermal ont été enregistrées sur le site Rainbow où d'abondants nuages de la crevette *Rimicaris exoculata* sont observés dans les dépressions des parois des cheminées. L'étude des relations entre le fluide et les organismes a été abordée à travers la quantification des métaux dans l'environnement immédiat des crevettes et dans leurs tissus, et en étudiant leur forme de stockage dans ces tissus. Notre travail met en évidence un fort enrichissement en métaux de l'environnement des crevettes. L'abondance relative de ces métaux est sous la dépendance de phénomènes de précipitation, d'adsorption et de redissolution oxydative. Leur bioaccumulation fluctue en accord avec leur biodisponibilité. Des dépôts externes sur les branchies et la cuticule de l'abdomen, qui s'ajouteraient au phénomène naturel de dégradation des métallothionéines dans les lysosomes, pourraient contribuer à leur forte accumulation sous forme de composés insolubles. Certaines questions soulevées par cette étude trouveront une réponse grâce à un meilleur conditionnement des échantillons biologiques, à des observations histologiques complémentaires, ainsi qu'à des dosages chimiques ou biochimiques. A l'avenir, la prise en compte de la distribution des métaux entre phases dissoute et particulaire dans l'eau environnant les organismes apparaît comme une nécessité pour évaluer la biodisponibilité des métaux.

Keywords: hydrothermal fluid, *Rimicaris exoculata*, metals, bioaccumulation, Rainbow.

Reçu le 1er juin 2001 ; accepté après révision le 5 décembre 2001.

Received 1st June 2001; accepted in revised form 5 December 2001.

Introduction

Deep-sea hydrothermal communities are dwelling in the interfacial zone where the hydrothermal fluid turbulently mixes with the bottom sea water. This fluctuating environment provides a periodical access to reduced chemical species (H_2S, CH_4) supplied by vent fluid and sea water oxidized compounds (O_2) both required for chemolithoautotrophic bacterial primary production. In other respects, this environment is enriched in potentially toxic species (sulphide and heavy metals) to which the organisms are exposed (Fisher, 1990; Childress & Fisher, 1992).

On the Mid-Atlantic Ridge (MAR), the Rainbow hydrothermal site (36°14'N, 2260-2650 m depth) is characterized by a tectonic control of the hydrothermal circulation in an ultramafic environment. The emitted fluids are subjected to a subsurface phase separation and present a high contribution of the brine phase enriched in metals and chloride. The metal concentrations (Cu, Fe, Mn, Zn) in the fluid are the highest observed in the MAR hydrothermal area (Desbruyères et al., 2000; Douville et al. (in press)). This metallic load produces an important total particle flux with a high sulphur contribution (Desbruyères et al., 2001).

The hydrothermal shrimps Alvinocarididae *Rimicaris exoculata* Williams & Rona 1986 have been observed in high densities (up to 2500 ind m^{-2}) on active chimney walls at several sites (Snake Pit, 23°N, TAG, 26°N, Broken Spur, 29°N, Lucky Strike, 37°N) on the MAR (Desbruyères & Segonzac, 1997). *Rimicaris exoculata* is present in the Rainbow field forming dense swarms (>3000 ind m^{-2}) in small depressions on the chimney walls. The measured temperature in the swarms ranges from 9 to 25°C (Desbruyères et al., 2000). *R. exoculata* is supposed to be heterotroph, grazing on free living bacteria present on sulphide particles (Van Dover et al., 1988), and likely on epibiotic bacteria (Segonzac et al., 1993).

In order to understand the fluid-organism relationship, we investigated the relative abundance of metals (Cd, Cu, Fe, Pb) in the water surrounding the animals and their accumulation by the shrimps. This approach focused on some essential (Cu, Fe, Mn, Zn) and one non-essential (Cd) metals. The pattern of their bioaccumulation in various anatomic parts of *R. exoculata* was compared to that of vent crustaceans from a fast spreading ridge (East Pacific Rise, EPR) to establish the hypothetical occurrence of a specific physiological adaptation to this metal-enriched environment.

Metallothioneins (MTs) were first reported in horse kidney by Margoshes and Vallee (1957). MTs are low molecular weight metalloproteins, with a high cystein content (30% of the 60-62 amino acid residues for mammalian MTs), a heat stable and non enzymatic nature, together with a strong affinity to bind Class B metal cations such as Ag, Cd, Cu, Hg and Zn. MTs are thought to be almost ubiquitous and have been reported for some 50 different aquatic invertebrates, three-quarters of which are molluscs or crustaceans (Langston et al., 1998). The primary function of MTs is dedicated to the homeostasis of essential metals such as Zn and Cu, but their involvement in the detoxication of non-essential metals (Ag, Cd, Hg) has been established (Roesijadi, 1992). Quantitative analyses of metallothioneins were performed in heat-denatured tissue homogenates to assess their relative involvement in metal regulation.

This study presents some results obtained in a highly dynamic medium enriched in potentially toxic compounds. It compares the composition of the metallic environment to the pattern of metal bioaccumulation in various anatomical parts of *R. exoculata* and the potential involvement of metallothioneins in the regulation of intracellular metals.

Materials and methods

Sampling

Water and shrimps were collected during the exploration of the Rainbow site (MARVEL cruise, 1997, chief scientists D. Desbruyères & A. M. Alayse, *N/O Atalante*) by the French submersible *Nautile*.
Water samples (n=23) were obtained using a new "multi-sampler", gathering four evacuated 200 ml titanium bottles and an autonomous temperature probe (Micrel,) in a frame manipulated by the submersible *Nautile*. The sampler is rinsed with HCl 0.1 N and deionized water before use. Sampling was done in and around the organisms swarms, on the smoker and diffuser walls. Temperature data were standardized against the value obtained by a standard reversing thermometre.

Sample preparation

The procedures used for water sample treatment are given Table 1.
Shrimps were dissected on board in order to isolate the gills, digestive gland and abdomen. These anatomical parts were stored in liquid nitrogen until analysis. The gills and abdomen of ten individuals were treated separately. The digestive glands of ten individuals were treated as three pools. After being weighed individual and pooled samples were homogenized at 4 °C using an electric potter and a Teflon pestle in a Tris (20 mM), NaCl (150 mM) buffer, pH 8.6 (4 ml g^{-1} soft tissue). The soluble and insoluble fractions were separated by centrifugation (30000*g*, 30 min, 4 °C). Aliquots of the supernatants (S1) were heated (15 min, 95 °C) and allowed to cool on ice (10 min). Heat-denaturable proteins were separated from heat-stable

Table 1. Sample treatment and analytical methods used for water analysis. DL°: detection limit; rsd*: relative standard deviation; **: total dissolved metal; #: adapted from King et al. (1991) and Chin et al. (1994).

Tableau 1. Traitement et méthodes d'analyse des échantillons d'eau. DL°: limite de détection; rsd*: écart-type relatif; **: métal total dissous; #: adapté de King et al. (1991) et de Chin et al. (1994).

Parameter method	Preservation	Analytical method	Remark
pH	analysis on board	Potentiometry electrode for sulphidic medium	25 °C TRIS buffer
ΣS ($H_2S + HS^-$)	analysis on board	Colorimetry (Fonselius 1983)	DL° = 0.5 µmol l^{-1} rsd * = 5%
Fe**	HNO_3 suprapur®, 20 µl in 20 ml, ambient T	Flow Injection Analysis Ferrozine #	sd 1.3 µM DL° = 0.3 µmol l^{-1}
Cu, Pb, Cd**	HNO_3 suprapur®, 20 µl in 20 ml, ambient T	Potentiometric Stripping Analysis (Riso et al., 1997)	rsd *= 6-8%

proteins by centrifugation of the heated supernatants (10000*g*, 15 min). Supernatants (S2) containing the heat-stable proteins, including metallothioneins, were stored at -20 °C until use.

Metal analysis

The analytical methods used for the analyses of water samples are given Table 1.
Concerning the biological samples, all labware was soaked in 10% hydrochloric acid, rinsed three times with deionized water, dried in a dessicator sheltered from atmospheric dusts. Supernatants (S1) and pellets (C1) from each anatomical part were heated (100 °C, one hour) with suprapure nitric acid (Carlo Erba) (1 ml per 1 ml S1; 1 ml per 0.5 g C1). After digestion, metals levels in these acidic solutions were determined, after dilution with deionized water, by flame (Cu, Fe, Mn, Zn) or electrothermal (Cd) atomic absorption spectrophotometry, using the Zeeman effect (Hitachi Z-8200 spectrophotometre).

Total metal levels in each anatomic part were calculated from quantities of trace metals in soluble and insoluble fractions determined previously. Standard addition analysis was performed in an isomedium. The analytical methods were validated by external intercalibrations (Mouneyrac et al., 1998). Metal levels were expressed as µmol g^{-1} wet weight.

Biochemical analysis

The amounts of total and heat-stable proteins were quantified respectively in the supernatants (S1) and (S2) using the Lowry et al. (1951) method.

In the heat-denatured cytosol (S2), the amount of metallothioneins was determined by differential pulse polarography (Olafson & Sim, 1979; Thompson & Cosson, 1984). Analyses were performed using a PAR 174A analyser and a EG&G PAR 303A static mercury drop electrode (SMDE).

The electrochemical detection of metallothioneins takes place in an ammoniacal electrolyte containing cobalt that catalyses the reduction of the cystein thiol groups (Brdicka, 1933). The standard addition method was used for calibration with rabbit liver metallothionein (Sigma Chemical Co) in the absence of invertebrate MT standard. The levels of metallothioneins were expressed as µg g^{-1} wet weight.

The polarographic quantification of metallothioneins is commonly used for bivalves (Bebianno & Langston, 1991; Pavicic et al., 1993). The specificity of this method was established by chromatography (Olafson & Olsson, 1991). It was validated by comparative assays versus other quantitative methods (Onosaka & Cherian, 1982; Hogstrand & Haux, 1989) and recommended (Summer & Klein, 1993).

Results

Microhabitat of Rimicaris exoculata

The microhabitat of *R. exoculata* exhibits a substantial hydrothermal influence characterized by a temperature increase, a pH decrease and ΣS enrichment compared to sea water (Table 2). The water surrounding the vent shrimps shows a high metallic enrichment regarding to sea water and a great variability as already observed by Sarradin et al. (1999). This variability is typical of a hydrothermal environment as stated for temperature (Johnson et al., 1988; Chevaldonné et al., 1991). The large concentration ranges observed for metals may also be linked to the potentially high heterogeneity of the particulate phase in the environment, considering that the sampling method did not discriminate between dissolved and particulate forms of the studied metals.

Table 2. Physical and chemical parameters of the water surrounding *Rimicaris exoculata* (12 to 15 samples), of bottom sea water, of the endmember* (concentrations from Douville et al. (in press)) and bottom sea water metallic concentrations from Li (1991).

*The endmember concentrations are the calculated concentrations of the Rainbow site pure hydrothermal fluid prior to dilution with sea water.

Tableau 2. Paramètres physico-chimiques de l'eau environnant *Rimicaris exoculata* (12 à 15 échantillons), de l'eau de mer de fond, du fluide pur* (concentrations d'après Douville et al. (sous presse)) et concentrations en métaux de l'eau de mer de fond d'après Li (1991).

*Les concentrations du fluide pur sont les concentrations calculées du fluide hydrothermal pur du site Rainbow avant sa dilution par l'eau de mer.

	T°C	pH	ΣS (µM)	Mn (µM)	Zn (µM)	Fe (µM)	Cu (µM)	Cd (nM)	Pb (nM)
min	4.7	6.3	0.4			58	0.14	1	4
max	25.0	7.5	22.0			1470	3.20	53	120
median	**11**	**7.0**	**4.4**			**323**	**0.96**	**9**	**30**
endmember*	365	2.8		2250	160	24000	140	130	148
bottom sea water	3.5	7.8	<0.1	0.001	0.028	0.004	0.003	0.7	0.013

The plots obtained for pH as a function of T °C (Fig. 1) and Fe as a function of pH (Fig. 2) are comparable with the endmember concentrations calculated by Douville et al. (2002). A clear decrease of iron concentration can be observed with increasing pH. Such trends are less clear for the other metals owing to their large variability of concentrations observed in the range of pH studied.

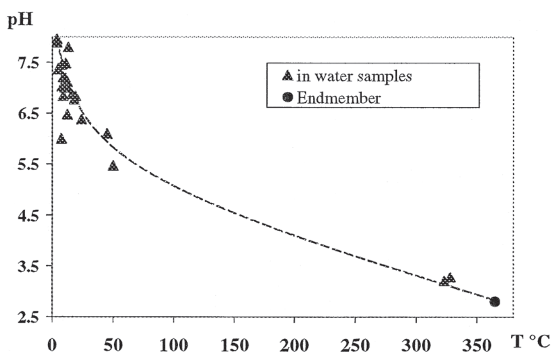

Figure 1. Variations of pH versus temperature in the water samples collected at the Rainbow hydrothermal site. The obtained outline is an approximation of the mixing curve. Endmember values from Douville et al. (in press).

Figure 1. Variations du pH en fonction de la température pour les différents échantillons d'eau collectés sur le site hydrothermal Rainbow. Le profil obtenu est une approximation de la courbe de dilution. Les valeurs utilisées pour la concentration du fluide pur sont tirées de Douville et al. (sous presse).

Manganese is often used as a conservative tracer of the fluid dilution staying under a dissolved form at least at short time scale of mixing (Mitra et al., 1994; James & Elderfield, 1996). We used this conservative behaviour and the data presented in table 2 (i.e. [Mn] = 2250 µM at 365 °C in the pure fluid ; [Mn] = 0.001 µM at 3.5 °C in sea water) to estimate the linear relationship between concentration of Mn and temperature. The following equation of the dilution curve was obtained:

$$Mn\ (\mu M) = 6.2T\ (°C) - 21.8$$

Using this equation, an estimation of the Mn concentration surrounding *R. exoculata* gives concentrations between 7 and 130 µM (median 46 µM).

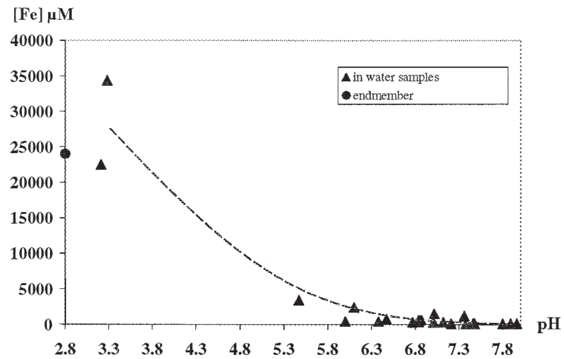

Figure 2. Variations of the iron (total) concentrations versus pH in the water samples collected at the Rainbow hydrothermal site. Endmember values from Douville et al. (in press).

Figure 2. Variations des concentrations en fer (total) en fonction du pH pour les différents échantillons d'eau collectés sur le site hydrothermal Rainbow. Les valeurs utilisées pour la concentration du fluide pur sont tirées de Douville et al. (sous presse).

A calculated dilution factor (relative ratio of median metal concentrations in the shrimp habitat *vs* metal concentration in the pure fluid) was roughly estimated using temperature as a conservative tracer (Table 3). The environment surrounding *Rimicaris exoculata* results from a mixture of 2% of hydrothermal fluid and 98% of sea water. An estimation of this relative dilution ratio is presented in Table 3 and gives indications on the non conservative behaviour of metals in the mixing zone. Temperature being considered as a conservative tracer of dilution, Cu and Fe appear to be impoverished (0.7 and 1.3% respectively), whereas Cd and Pb are enriched (6.9 and 20% respectively) compared to the conservative dilution ratio obtained with temperature (2%).

Metal bioaccumulation

In accordance with the high particle load, the gills and the digestive gland showed high levels of metals (Table 4). Metals may be present on the surface of the gills associated with deposits. In the digestive gland they may reflect the ingestion of metal-enriched particles.

Table 3. Ratio of the median concentration of metal in the water surrounding *Rimicaris exoculata* to the corresponding concentration calculated for the endmember (Table 2).

*This ratio is corrected for sea water temperature $R = (T_{Rimicaris}-T_{seawater})/ T_{endmember}$.

Tableau 3. Rapport entre la concentration médiane en métal dans l'eau environnant *Rimicaris exoculata* et la concentration correspondante calculée pour le fluide pur (Tableau 2).

*Ce rapport a été corrigé en tenant compte de la température de l'eau de mer $R = (T_{Rimicaris}-T_{eau\ de\ mer})/ T_{fluide}$.

	Fe	Cu	Cd	Pb	T*
Ratio %	1.3	0.7	6.9	20	2

Table 4. Mean level (standard deviation) of metals within anatomical parts of *Rimicaris exoculata*. Results are expressed as µmol g^{-1} wet weight. For the gills and abdomen, the organs of ten individuals were analysed separately. For the digestive gland, the organs of ten individuals were analysed as three pools.

Tableau 4. Teneur moyenne (écart-type) en métaux de différentes parties anatomiques de *Rimicaris exoculata*. Les résultats sont exprimés en µmol g^{-1} de poids frais. Les branchies et les abdomens de dix crevettes ont été analysés individuellement. Les glandes digestives de dix crevettes ont été regroupées en trois échantillons pour l'analyse.

	Mn	Fe	Cu	Zn	Cd
Gills	0.316 (0.18)	25.5 (11.4)	1.20 (0.60)	17.9 (5.0)	0.014 ($4\ 10^{-3}$)
Digestive gland	0.076 (0.009)	24.6 (1.8)	0.61 (0.21)	5.7 (1.7)	$3.5\ 10^{-3}$ ($9\ 10^{-4}$)
Abdomen + cuticle	0.039 (0.026)	3.1 (1.86)	0.26 (0.08)	1.5 (1.1)	$1.1\ 10^{-3}$ ($9\ 10^{-4}$)

The levels of metals in the anatomical parts reflect their concentration in the environment, apart from Mn which is predominant over Cu and Zn in the fluid, whereas Cu and Zn are predominant over Mn in the anatomical parts.

The levels of iron were relatively high in the tissues owing to Fe abundance in the fluid (Table 2). Most of Fe (40% to 90%) forms particulate sulphide settling within the first minutes of mixing (Mottl & MacConachy, 1990; Field & Sherrell, 2000). Consequently Fe surrounding organisms may be predominantly in the particulate phase, as iron sulphides or iron hydroxides, newly formed in the mixing zone, and really abundant on all surfaces at the Rainbow site.

While the concentrations of zinc and copper in the endmember (Table 2) were in the same order of magnitude, their relative abundance in *Rimicaris exoculata* tissues was quite different (ca. 10 to 20 fold higher for zinc). The very high bioavailability of zinc is underlined by its equal level to iron within the gills, in contrast to the higher concentration of iron (ca. 100 fold) in the endmember fluid.

The levels of cadmium (a non essential metal) within anatomical parts (Table 4) were relatively low in comparison with those of essential metals (copper, zinc), owing to Cd lesser abundance within *R. exoculata* surrounding sea water (Table 2).

The levels of manganese were low in the shrimps in contrast to the high concentrations observed in the fluid (Table 2).

The ratios, mean levels of metals within anatomical parts vs. median concentration in the environment ("concentration factor": CF), are presented in Fig. 3. The calculated CFs for Mn were very low (with a maximum reaching 0.007 for the gills) reflecting a lack of bioaccumulation by the shrimp. Iron seems poorly bioaccumulated too, since the maxima were around 0.080 for the gills and the digestive gland (ca. tenfold the higher value recorded for Mn). On the contrary, copper and cadmium are bioaccumulated by every studied anatomical part showing CFs over 0.100. In the gills the CFs for both metals reached very high values ranging from 1.200 to 1.570. The general trend is a decrease of the ratio from the gills to the abdomen, in relation with the exposure of the organ to the abundant flow of metal-loaded particles (external vs. internal organs) and the involvement of the organ in metal metabolism (gills and digestive gland vs. muscle). However this trend varies according to the metal itself and its bioavailability.

Metal partitioning

A metal under a soluble form is susceptible to circulate through the body and to disturb essential metabolic functions. Stored as a mineral concretion its neutralization is presumably achieved. We quantified the insoluble and soluble forms of metals respectively in the pellets (C1) and the supernatants (S1) obtained after the centrifugation of tissue homogenates (Table 5). The manganese could not be detected in the supernatants by flame atomic absorption spectrophotometry.

With the exception of iron and copper, equally abundant under soluble and insoluble forms within the digestive gland, and of copper in the abdomen, metals were essentially under insoluble forms. Except for iron, we noticed a higher ratio of metals associated with soluble compounds in the gills (external organ) compared to the abdomen.

Biochemical approach

The levels of total proteins (PS1), heat-stable proteins (PS2), and metallothioneins (MTs) and their relative ratios in tissues of the shrimp *Rimicaris exoculata* are presented Table 6.

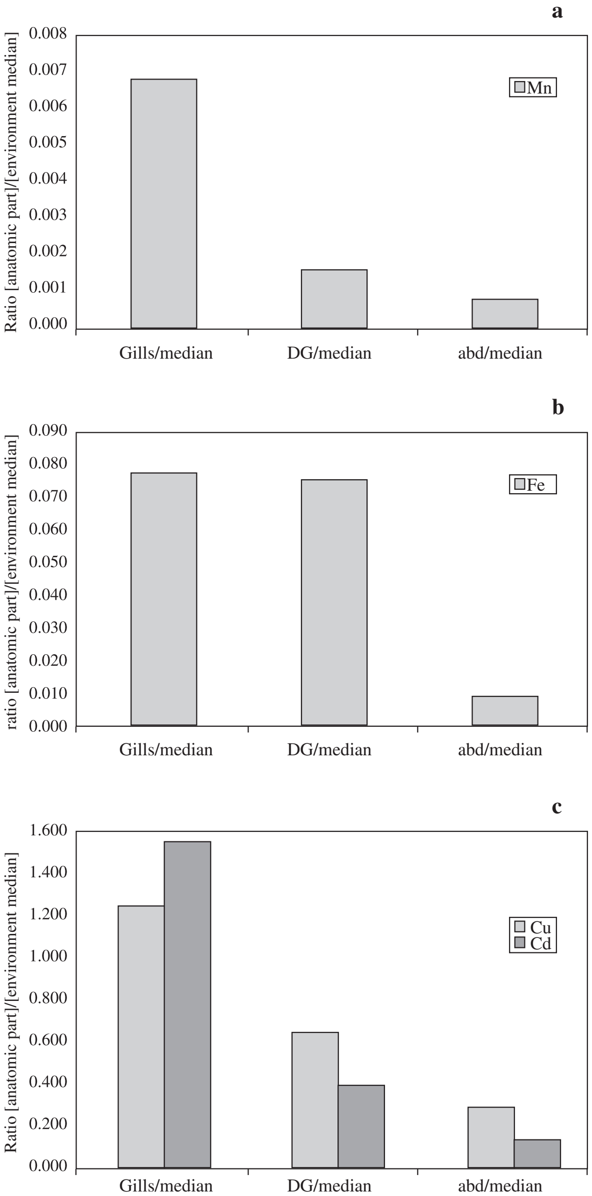

Figure 3. Ratio of the mean level of metal (**a**: Mn; **b**: Fe; **c**: Cd and Cu) within different anatomical parts (gills; (DG) digestive gland; (abd) abdomen) of *Rimicaris exoculata* to the median concentration of metal in the water surrounding the shrimp ("concentration factor"). For the manganese the median concentration was calculated using the following dilution equation: Mn (µM) = 6.2T (°C) – 21.8.

Figure 3. Rapport entre la teneur moyenne en métal (**a** : Mn ; **b** : Fe ; **c** : Cd et Cu) des différentes parties anatomiques (branchies; (DG) glande digestive; (abd) abdomen) de *Rimicaris exoculata* et la concentration médiane en métal dans l'eau environnant les crevettes ("facteur de concentration"). Pour le manganèse la concentration médiane a été calculée en utilisant l'équation de dilution suivante : Mn (µM) = 6,2T (°C) – 21,8.

In connection with its metabolic importance, the digestive gland showed the highest level of total proteins (PS1), heat-stable proteins (PS2) and metallothioneins (MTs).

Table 5. Ratio (standard deviation) of metals under soluble forms (S1) in three anatomical parts of *Rimicaris exoculata*. The tissues were homogenized in a Tris/NaCl buffer then centrifuged (30000*g*, 30min, 4 °C). The supernatant (S1) contained the soluble forms of metals. The ratios are expressed as % of the total amount of metal in the sample. For the number of analysed samples : see Table 4.

Tableau 5. Proportion (écart-type) des métaux sous forme soluble (S1) dans trois parties anatomiques de *Rimicaris exoculata*. Les tissus ont été homogénéisés dans un tampon Tris/NaCl puis centrifugés. Le surnageant (S1) contient les métaux sous forme soluble (30000*g*, 30min, 4 °C). Les proportions sont exprimées en pourcentage de la quantité totale de métal présente dans l'échantillon. Pour le nombre d'échantillons analysés voir le Tableau 4.

	Fe	Cu	Zn	Cd
Gills	2.4 (1.3)	21.2 (8.8)	0.7 (0.2)	0.6 (0.4)
Digestive gland	47.5 (7.0)	46.7 (10.4)	8.3 (3.0)	7.5 (2.7)
Abdomen	8.8 (7.8)	75.8 (7.8)	11.7 (8.2)	15.6 (10.0)

Table 6. Mean level (standard deviation) of total proteins (PS1), heat-stable proteins (PS2), and metallothioneins (MTs) expressed as µg mg^{-1} wet weight, and their relative ratios (expressed as percentage) in three anatomical parts of *Rimicaris exoculata*. For the number of analysed samples: see Table 4.

Tableau 6. Teneur moyenne (écart-type) en protéines totales (PS1), protéines thermostables (PS2), et métallothionéines (MTs) exprimées en µg mg^{-1} de poids frais, et leur relative abondance (exprimée en pourcentage) dans trois parties anatomiques de *Rimicaris exoculata*. Pour le nombre d'échantillons analysés voir le Tableau 4.

	PS1	PS2	PS2/PS1	MTs	MTs/PS1	MTs/PS2
Gills	28.44 (5.3)	7.47 (1.4)	27 (6)	0.255 (0.10)	0.9 (0.4)	3.4 (1.0)
Digestive gland	60.61 (4.2)	44.01 (5.0)	73 (11)	3.012 (0.82)	5.0 (1.6)	6.8 (1.0)
Abdomen	26.57 (6.7)	5.31 (1.4)	20 (0)	0.072 (0.02)	0.3 (0.2)	1.5 (0.8)

Discussion

Cu and Fe, as well as Cd, Pb, Co, Hg and Zn are removed from the medium by precipitation processes within the mixing zone as they are known to form predominantly insoluble precipitates with sulphides in plumes or on chimneys, and on conduit surfaces (Von Damm et al.,1983; Trefry & Trocine, 1985; Von Damm, 1995). James & Elderfield (1996) studied the dissolved and particulate trace metals in the non buoyant plumes of the TAG and Snake Pit hydrothermal sites (MAR), and found up to 50% Fe or Cu in the <0.4 µm fraction. Mottl & Mac Conachy (1990) and Field & Sherrell (2000) found that in the plume, most of Fe (40 to 90%) will form particulate sulphides settling within the first minutes of mixing. The loss of particulate Fe and Cu observed in this study can be attributed to particle fall out in the early stage of plume mixing. Cd and Pb may be involved in this process to a lesser extent or could form smaller particles more likely to stay in the environment. James & Elderfield (1996) showed that Cd in the plume is mostly in the particulate phase, even with depleted dissolved concentrations compared to sea water. Cd is clearly scavenged from the plume by particles which could undergo redissolution; Cd can also be released from these particles (James & Elderfield, 1996). The oxidative redissolution of sulphide particles can explain the distinct enrichment, leading to an increased availability of these metals, compared to the conservative dilution ratio.

This first approach of the metallic load surrounding *R. exoculata* stresses the net metal enrichment around the organisms. The mixing zone is chemically active with large changes in pH, temperature and metal concentrations. Precipitation, scavenging and oxidative redissolution processes are controlling the metal concentrations and emphazize the necessity of a further study on the distribution of metals between dissolved and parciculate fractions. This will constitute a first step towards the estimation of the bioavailability of metals.

Manganese, though largely present in the environment and probably predominant in the dissolved phase (Mitra et al., 1994) is not bioaccumulated by the organism. Iron shows also a low concentration factor, though it is the preponderant metal in the fluid. However it should be predominantly present in the particulate phase (Mottl & MacConachy, 1990; Field & Sherrell, 2000). Conversely, cadmium and copper are characterized by a high concentration factor suggesting an important bioavalability. The bioaccumulation of Cu can be related to the large amounts (mean level : 2900 mM of free Cu) found in the haemolymph of *Rimicaris exoculata* (Chausson, 2001) since copper is also the component metal of haemocyanin. Cadmium is enriched compared to the conservative dilution ratio (Table 3) and can be released from the sulphide particles to the dissolved fraction enhancing its bioavailability (James & Elderfield, 1996). Zinc levels in the gills and the digestive gland of *R. exoculata* were higher than those observed earlier for the same organs within the hydrothermal crab *Bythograea thermydron* Williams, 1980, from the EPR according to re-calculated data from Cosson & Vivier (1997) (gills: 17.79 ± 4.97 µmol g^{-1} w.w.; digestive gland: 5.68 ± 1.70 µmol g^{-1} w.w.) On the contrary the level of copper in the digestive gland was lower (gills: 1.21 ± 0.60 µmol g^{-1} w.w.; digestive gland: 0.61 ± 0.20 µmol g^{-1} w.w.). Given the protective role of the cuticle, the decapods have a low metal uptake, permeability being restricted to the gills (Rainbow, 1998). The observed difference between *Rimicaris exoculata* and *Bythograea thermydron* metal burdens could be attributed to anatomical characteristics (surface areas and exposure of their gills) and feeding habits (bacteria grazer vs. carnivorous). Muscle is generally considered as a not particularly metal rich tissue (Rainbow, 1998). Our observations for *R. exoculata* abdomen have to take into account eventual deposits on the cuticle.

The bioaccumulation of metals under insoluble forms is often observed when studying invertebrates with a high global content of metals (Bustamante, 1998). However, concerning the gills (Cd and Zn) and the abdomen (Cd, Fe and Zn) we could not reject the hypothesis of an external deposit of particulate material enriched with metals. *Rimicaris exoculata* showed a ratio of soluble metals, higher within the digestive gland (internal organ) than within the gills (exposed organ), as already noticed for *Bythograea thermydron* (Cosson & Vivier, 1997). Within the digestive gland the insoluble form of metals originate from the digestive tract by pinocytosis, or from the degradation of metalloproteins in lysosome-like vesicules. Excretion of trace metals in detoxified granular forms, from the epithelium of midgut, including the digestive gland, is not uncommon in crustaceans (Rainbow, 1998). A microscopical study would be necessary to investigate an hypothetical storage of metals under mineral concretions inside the tissues of the gills. The effective participation of external deposits to the amount of metals registered for the abdomen should be investigated on samples without any cuticle. In comparison with the data from *B. thermydron* (Cosson & Vivier, 1997) *R. exoculata* showed lower ratios of soluble metals within the gills and the digestive gland, with the exception of copper within the gills. The presence of copper under soluble form in the gills, digestive gland and abdomen can again be related to the large concentrations of copper found in the haemolymph of *R. exoculata* and to its importance as a haemocyanin component (Chausson, 2001).

In this study, particulate and dissolved metals were not discriminated. The main uptake route for particulate metals comes from food, while dissolved metals are able to enter the organism by diffusion through membranes in contact with the environment. In the future, our efforts will focus on the separation of particulate and dissolved metals, the latter being generally more bioavailable for aquatic organisms and more harmful.

The relative abundance of heat-stable proteins in various tissues of *Rimicaris exoculata* were not identical to that published earlier for the crab *Bythograea thermydron* (Cosson & Vivier, 1997). Heat-stable proteins were very abundant in the gills of the crab (88% of total proteins) in comparison with the gills of the shrimp (27%); on the contrary, in the digestive gland we noted 73% of total proteins for the shrimp and 37% for the crab. The levels of metallothioneins in the gills and the digestive gland of both species were close. The level of metallothioneins recorded for *Rimicaris exoculata* abdomen was low owing to the moderate contribution of that kind of tissue (muscle) to metal metabolism in general. The ratios of metallothioneins versus total or heat-stable proteins were higher in the digestive gland than in the gills, in relation to a higher ratio of soluble metals, taking into account that external deposits on the gills could result in a bias.

Metallothioneins are not only induced in response to an external trace metal challenge, but are also involved in the homeostasis of zinc and copper. In crustaceans these essential metals are mobilized and immobilized during the moult cycle (Engel & Brouwer, 1991; 1993). The relative abundance of metal under insoluble forms may be related to the regular lysosomal breakdown of metallothioneins (Rainbow, 1998).

Conclusion

This first approach of the relationship between *Rimicaris exoculata* and the metal content of its environment emphasizes the following points:

a net metal enrichment around the organisms. The mixing zone is chemically active with large changes in pH, temperature and metal concentrations. Precipitation, scavenging and oxidative redissolution processes are controlling the concentrations of metals;

the bioaccumulation of metals in the tissues of *R. exoculata* varies according to the metal and can be related to its bioavailability in the surrounding water;

metals are abundant as insoluble forms in the tissues but the hypothesis of external deposits on the gills and the cuticle of the abdomen cannot be rejected;

the involvement of the digestive gland in metal metabolism and the moderate participation of the muscular tissue (abdomen) are confirmed;

the gills, a site of exchanges with the ambient medium, play an important part in the intracellular metal regulation (uptake, storage, excretion); that will be clarified when data about hypothetical external minerals deposits at their surface will be available.

Improvements concerning the preparation of the biological material and the use of a complementary histological technique are needed to answer the questions emerging from our work. The necessity of studying the distribution of metals between dissolved and particulate fractions, a first step towards the evaluation of their bioavailability, is underlined.

Chemical and biochemical analyses are useful tools, necessary to describe the state of the vent organisms in relation with their metallic environment, but there is a lack of information concerning the ability of these organisms to handle the bioaccumulated metals. The development of controlled contamination experiments will provide useful informations.

Acknowledgements

This research has been undertaken in the framework of the AMORES project. We acknowledge the support from the European Commission's Marine Science and Technology Program (MAST III) under contract MAS3-CT95-0040. The diving cruise MARVEL was supported by IFREMER, France.

References

Bebianno M.J. & Langston W.J. 1991. Metallothionein induction by *Mytilus edulis* exposed to cadmium. *Marine Biology*, **108:** 91-96.

Brdicka R. 1933. Polarographic studies with the dropping mercury cathode. XXXI. A new test for proteins in the presence of cobalt salts in ammoniacal solutions of ammonium chloride. *Collection of Czech Chemical Communications*, **5:** 122-128.

Bustamante P. 1998. *Bioaccumulation des éléments traces chez les mollusques céphalopodes et bivalves pectinidés. Implication de leur biodisponibilité pour le transfert vers les prédateurs*. Thèse de l'Université de La Rochelle 291 pp.

Chausson F. 2001. *Adaptation au milieu hydrothermal profond : étude comparative de l'écophysiologie respiratoire des crustacés décapodes des dorsales Pacifique et Atlantique*. Thèse de l'Université P. & M. Curie (Paris VI) 184 pp.

Chevaldonné P., Desbruyères D. & Le Haître M. 1991. Time-series of temperature from three deep-sea hydrothermal vent sites. *Deep-Sea Research*, **38:** 1417-1430.

Childress J.J. & Fisher C.R. 1992. The biology of hydrothermal vent animals : physiology, biochemistry and autotrophic symbioses. *Oceanography and Marine Biology an Annual Review*, **30:** 337-441.

Chin C.S., Coale K.H., Elrod V.A., Johnson K.S., Massoth G.J. & Baker E.T. 1994. In situ observations of dissolved iron and manganese in hydrothermal plumes, Juan de Fuca Ridge. *Journal of Geophysical Research*, **99:** 4969-4984.

Cosson R.P. & Vivier J.P. 1997. Interactions of metallic elements and organisms within hydrothermal vents. *Cahiers de Biologie Marine*, **38:** 43-50.

Desbruyères D., Almeida A, Biscoito M., Comtet T., Khripounoff A., Le Bris N., Sarradin P.M., & Segonzac M. 2000. A review of the distribution of hydrothermal vent communities along the Northern Mid-Atlantic Ridge: Dispersal vs environmental controls. *Hydrobiologia*, **440:** 201-216.

Desbruyères D., Biscoito M., Caprais J.C., Colaço A., Comtet T., Crassous P., Fouquet Y., Khripounoff A., Le Bris N., Olu K., Riso R., Sarradin P.M., Segonzac M. & Vangriesheim A. 2001. Variations in deep-sea hydrothermal vent communities on the Mid-Atlantic Ridge near the Azores plateau. *Deep-Sea Research*, **48:** 1325-1346.

Desbruyères D. & Segonzac M. 1997. Handbook of deep-sea hydrothermal vent fauna. Éditions IFREMER, Brest : 279p.

Douville E., Charlou J.L., Oelkers E.H., Bienvenu P., Jove Colon C.F., Donval J.P., Fouquet Y., Prieur D. & Appriou P. (in press). The Rainbow vent fluid (36°14'N, MAR): the influence of ultramafic rocks and phase separation on trace metal content in Mid-Atlantic Ridge hydrothermal fluids. *Chemical Geology*,

Engel D.W. & Brouwer M. 1991. Short-term metallothionein and copper changes in blue crab at ecdysis. *Biological Bulletin*, **180:** 447-452.

Engel D.W. & Brouwer M. 1993. Crustaceans as models for metal metabolism: I. Effects of the molt cycle on blue crab metal metabolism and metallothionein. *Marine Environmental Research*, **35:** 1-5.

Field M.P. & Sherrell R.M. 2000. Dissolved and particulate Fe in a hydrothermal plume at 9°45'N, East Pacific Rise: Slow Fe (II) oxidation kinetics in Pacific plumes. *Geochimica et Cosmochimica Acta*, **64:** 619-628.

Fisher C.R. 1990. Chemoautotrophic and methanotrophic symbioses in marine invertebrates. *Aquatic Sciences*, **2:** 399-436.

Fonselius S.H. 1983. Determination of hydrogen sulfide. In: *Methods of sea-water analysis* (K. Grasshof ed), pp.73-84. Verlag Chemie: Kiel.

Hogstrand C. & Haux C. 1989. Comparison of polarography and radioimmunoassay for the quantification of metallothionein in perch (*Perca fluviatilis*). *Marine Environmental Research*, **28:** 187-190.

James R.H. & Elderfield H. 1996. Dissolved and particulate trace metals in hydrothermal plumes at the Mid-Atlantic Ridge. *Geophysical Research Letters*, **23:** 3499-3502.

Johnson K.S., Childress J.J. & Beelher C.L. 1988. Short time temperature variability in the Rose Garden hydrothermal vent field: an unstable deep-sea environment. *Deep-Sea Research*, **35:** 1711-1721.

King D.W., Lin J. & Kester D.R. 1991. Spectrophotometric determination of iron(II) in seawater at nanomolar concentrations. *Analytica Chimica Acta*, **247:** 125-132.

Li Y.H. 1991. Distribution patterns of the elements in the ocean: A synthesis. *Geochimica et Cosmochimica Acta*, **55:** 3223-3240.

Langston W.J., Bebianno M.J. & Burt G.R. 1998. Metal handling strategies in molluscs. In : *Metal Metabolism in Aquatic Environnements* (W.J. Langston & M.J. Bebianno eds), pp. 219-283. Chapman & Hall: London.

Lowry O.H., Rosebrough N.J., Farr A.L. & Randall R.J. 1951. Protein measurement with the folin phenol reagent. *Journal of Biology and Chemistry*, **193:** 265-275.

Mitra A., Elderfield H. & Greaves M.J. 1994. Rare earth elements in submarine hydrothermal fluids and plumes from the Mid-Atlantic Ridge. *Marine Chemistry*, **46:** 217-235.

Margoshes M. & Vallee B.L. 1957. A cadmium protein from equine kidney cortex. *Journal of the American Chemical Society*, **79:** 4813-4819.

Mottl M. J. & MacConachy T.F. 1990. Chemical processes in buoyant plumes on the EPR near 21 N. *Geochimica et Cosmochimica Acta*, **54:** 1911-1927.

Mouneyrac C., Amiard J.C. & Amiard-Triquet C. 1998. Effects of natural factors (salinity and body weight) on cadmium, copper, zinc and metallothionein-like protein levels in resident populations of oysters *Crassostrea gigas* from a polluted estuary. *Marine Ecology Progress Series*, **162:** 125-135.

Olafson R.W. & Olsson P.E. 1991. Electrochemical detection of metallothionein. *Methods in Enzymology*, **205:** 205-213.

Olafson R.W. & Sim R.G. 1979. An electrochemical approach to quantification and characterization of metallothioneins. *Analytical Biochemistry*, **100:** 343-351.

Onasaka S. & Cherian M.G. 1982. Comparison of metallothionein determination by polarographic and cadmium-saturation methods. *Toxicology and Applied Pharmacology*, **63:** 270-274.

Pavicic J., Raspor B. & Martincic D. 1993. Quantitative determination of metallothionein-like proteins in mussels. Methodological approach and field evaluation. *Marine Biology*, **115:** 435-444.

Rainbow P.S. 1998. Phylogeny of trace metal accumulation in crustaceans. In : *Metal Metabolism in Aquatic Environnements* (W.J. Langston & M.J. Bebianno eds), pp. 285-320. Chapman & Hall: London.

Riso R.D., Le Corre P. & Chaumery C.J. 1997. Rapid and simultaneous analysis of trace metals (Cu, Pb, and Cd) in seawater by potentiometric stripping analysis. *Analytica Chimica Acta*, **351:** 83-89.

Roesijadi G. 1992. Metallothioneins in metal regulation and toxicity in aquatic animals. *Aquatic Toxicology*, **22:** 81-114.

Sarradin P.-M., Caprais J.C., Riso R., Kerouel R. & Aminot A. 1999. Chemical environment of the hydrothermal mussels communities in the Lucky Strike and Menez Gwen vent fields, MAR. *Cahiers de Biologie Marine*, **40:** 93-104.

Segonzac M., de Saint Laurent M. & Casanova B. 1993. L'énigme du comportement trophique des crevettes Alvinocarididae des sites hydrothermaux de la dorsale médio atlantique. *Cahiers de Biologie Marine*, **34:** 535-571.

Summer K.H. & Klein D. 1993. Quantification of metallothionein in biological materials. In : *Metallothionein III* (K.T. Suzuki, N. Imura & M. Kimura eds), pp. 75-86. Birkhäuser Verlag: Basel.

Thompson J.A.J. & Cosson R.P. 1984. An improved electrochemical method for the quantification of metallothioneins in marine organisms. *Marine Environmental Research*, **11:** 137-152.

Trefry J.H. & Trocine R.P. 1985. Iron and copper enrichment of suspended particles in dispersed hydrothermal plumes along the mid Atlantic ridge. *Geophysical Research Letters*, **12:** 506-509.

Van Dover C.L., Fry B., Grassle J.F., Humphris S. & Rona P.A. 1988. Feeding biology of the shrimp *Rimicaris exoculata* at hydrothermal vents on the Mid-Atlantic Ridge. *Marine Biology*, **98:** 209-216.

Von Damm K.L., Grant B. & Edmond J.M. 1983. Preliminary report on the chemistry of hydrothermal solutions at 21°N, east Pacific rise. In: *Hydrothermal Processes at Seafloor Spreading Centers* (P.A. Rona, K. Boström, L. Laubier & K.L.J. Smith eds), pp. 369-389. Plenum press**:** New York.

Von Damm K.L. 1995. Controls on the chemistry and temporal variability of seafloor hydrothermal fluids. *American Geological Union*, **91**: 222-247.

Williams A.B. 1980. A new crab family from the vicinity of submarine thermal vents on the Galapagos Rift (Crustacea: Decapoda: Brachyura). *Proceedings of the Biological Society of Washington*, **93:** 443-472.

Williams A.B. & Rona P.A. 1986. Two new caridean shrimps (Bresiliidae) from a hydrothermal field on the Mid-Atlantic Ridge. *Journal of Crustacean Biology*, **6:** 446-462.