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Speciation of dissolved copper within an active hydrothermal edifice on the Lucky Strike vent field (MAR, 37°N)

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Abstract:

The objective of this study was to determine the concentrations of different fractions of dissolved copper (after filtration at 0.45 µm) along the cold part of the hydrothermal fluid-seawater mixing zone on the Tour Eiffel edifice (MAR). Dissolved copper was analyzed by stripping chronopotentiometry (SCP) after chromatographic C18 extraction. Levels of total dissolved copper (0.03 to 5.15 µM) are much higher than those reported for deep-sea oceanic waters but in accordance with data previously obtained in this area. Speciation measurements show that the hydrophobic organic fraction (C18Cu) is very low (2 ± 1%). Dissolved copper is present mainly as inorganic and hydrophilic organic complexes (nonC18Cu). The distribution of copper along the pH gradient shows the same pattern for each fraction. Copper concentrations increase from pH 5.6 to 6.5 and then remain relatively constant at pH > 6.5. Concentrations of oxygen and total sulphides demonstrate that the copper anomaly corresponds to the transition between suboxic and oxic waters. The increase of dissolved copper should correspond to the oxidative redissolution of copper sulphide particles formed in the vicinity of the fluid exit. The presence of such a secondary dissolved copper source, associated with the accumulation of metal sulphide particles, could play a significant role in the distribution of fauna in the different habitats available at vents.

Keywords: Dissolved copper; Speciation; Hydrothermal vent; Mid Atlantic Ridge

1. Introduction

The circulation of seawater through fractured rocks at mid oceanic ridges produces important exchanges between lithosphere and hydrosphere. The resulting hydrothermal vents provide hot, reduced and acidic fluids which contain high amounts of various mineral compounds, including hydrogen sulphide, methane, carbon dioxide and many metals such as iron, manganese and copper, (Von Damm et al., 1998; Charlou et al., 2000; Douville et al., 2002). The peculiar and highly productive dense fauna encountered close to hydrothermal vents is not dependant on photosynthesis but rely on microbial chemosynthetic primary producers using reduced chemicals present in the hydrothermal fluid (Childress and Fisher, 1992). Hydrothermal vent fauna is periodically bathed within a variable mixture of hydrothermal fluid and seawater. This environment is chemically highly reactive with the coexistence of oxidized and reduced chemical species (Luther et al., 2001). Steep temperature and chemical gradients are encountered produced by the turbulent mixing of the hot fluid and the cold seawater (Le Bris et al., 2006). The hydrothermal fluid provides to the vent fauna at the same time the necessary energy sources such as methane and hydrogen sulphide (Urcuyo et al., 2003), but also potential stressors or “toxic” compounds such as heavy metals (Douville et al., 2002; Sarradin et al., 2008), high temperature (Shillito et al., in press) or radionuclides. The understanding of the chemical and biological processes controlling the composition of this mixing zone is a necessary step in the study of the functioning of the whole ecosystem (Sarrazin et al., 2006b). The study of trace metals along the hydrothermal fluid-seawater mixing zone is of highly importance because some of these elements and particularly copper can be both essential and toxic for many biological species (Cosson and Vivier, 1997). Mytilids mussels, as well as clams and vestimentiferan tube worms, living in the vent environment accumulate large amounts of metals with surprisingly no evident deleterious

59 effects (Geret et al. 1998, Ruelas-Inzunza et al. 2003 and 2005, Cosson and Vivier 1997,
60 Cosson et al., 2008). To cope with their harmful environment, they have developed efficient
61 mechanisms against the toxicity of heavy metals including their intracellular sequestration as
62 non toxic granules, or the production of metallothioneins and antioxidants (Cosson and
63 Vivier, 1997; Bebianno et al., 2005).
64 Studies exploring the behaviours of metals after their emission in the hydrothermal fluid were
65 mainly focussed on the plume (Trefry and Trocine, 1985; Feely et al., 1990 and 1994; James
66 and Elderfield, 1996). A few authors have started to describe the characteristics of total metal
67 concentrations in the habitats of the hydrothermal fauna (Sarradin et al., 1999; Desbruyères et
68 al., 2001; Geret et al., 2002; Di Meo-Savoie et al., 2004). The main observations arising from
69 these papers presenting concentrations of total metals are the enrichment of this part of the
70 mixing zone accounting for the hydrothermal input and the large variability in the obtained
71 data. However, studies on speciation are necessary to improve our knowledge on metal
72 behaviour and availability in hydrothermal environments. First trials were done on a 2-µm
73 fractionation for Cu, Zn, Cd and Pb in hydrothermal water samples from the EPR (Sarradin et
74 al., 2008). In particular, the results showed that particulate (> 2 µm) and dissolved (< 2 µm)
75 Cu were not following a conservative behaviour during the mixing of hydrothermal fluid and
76 seawater.
77 In natural waters, copper coexists under different chemical species such as free hydrated
78 cations $[Cu(H_2O)_6]^{2+}$, as well as inorganic complexes ($CuOH^-$, $CuCO_3$…) and organic
79 complexes (CuL). Recently, Sander et al. (2007) reported for the first time the important role
80 of organic ligands to control the copper speciation in hydrothermal systems. However, the
81 exact nature and evolution of the various copper species along the hydrothermal fluid-
82 seawater mixing zone are still unknown.

The study reported here was carried out in the Tour Eiffel hydrothermal edifice located on the Lucky Strike field on the Mid Atlantic Ridge. Dissolved copper (not retained on a 0.45 µm filter) was analyzed by stripping chronopotentiometry (SCP) after solid-liquid chromatographic C_{18} extraction (Mills and Quinn, 1981). From this extraction, two fractions are obtained: (i) the non-polar hydrophobic organic one ($C_{18}Cu$) is a subset of the organically-complexed pool and may greatly affect the metal bioavailability (Elbaz-Poulichet et al., 1994); (ii) the second fraction consists of inorganic and hydrophilic organic complexes ($nonC_{18}Cu$). It is worth noting that the C_{18} method has been extensively used for studies on estuarine, coastal and oceanic waters (e.g. Mills and Quinn, 1981; Paulson et al., 1994; Elbaz-Poulichet et al., 1994; Yoon et al., 1999; El Sayed and Aminot, 2000; Waeles et al., 2004 and 2005) but has never been implemented for hydrothermal systems. Our aim was thus to determine the concentrations of not retained copper and of its various species on an active hydrothermal edifice and also to investigate their changes along the cold part of the hydrothermal fluid-seawater mixing zone.

2. Material and methods

2.1 study area

This study was conducted during the MoMARETO cruise (Sarrazin et al., 2006) held on the French oceanographic vessel “Pourquoi pas?” with the ROV Victor 6000 in 2006. It focussed on the active hydrothermal edifice Tour Eiffel within the Lucky Strike vent field (37°17,29' N, 32°16,45' W) on the Mid Atlantic Ridge (Figure 1). Lucky Strike is one of the largest known active hydrothermal vent fields. The vent sites are distributed around a large lava lake at depths varying from 1650 to 1750 m (Fouquet et al., 1995). Faunal communities are dominated by extensive mussel beds of *Bathymodiolus azoricus* partially covered by visible microbial mats. The vicinity of active high-temperature chimneys, flanges and cracks

are colonized by shrimp assemblages (Desbruyères et al., 2001). On the Tour Eiffel edifice, 4 or 5 chimneys are venting a hot fluid with a temperature of up to 325°C, concentrations of Fe ranging from 595 to 704 µM and H_2S concentrations of 2100-2500 µM (Charlou et al., 2000). Twelve sampling units were chosen on different sides and at different altitudes on the 11-m tall sulphide edifice in order to have a representative overview of the chemical conditions over the faunal assemblages (Figure 2).

2.2 Sampling and sample treatment

Forty two water samples were collected on the 12 sampling units (2-5 samples per station; Figure 2) using the "PEPITO" sampler of the ROV Victor 6000. PEPITO is a new water sampling device qualified up to 6000 m depth (Sarradin et al., 2007). It can collect up to 23 water samples in 200 mL titanium/PEEK bottles. All the materials used for the transfer or storage of the samples are chemically inert (PEEK, Titanium, Viton). The manifold is designed to minimize contamination between samples with very low dead volumes. The samples were pumped using a titanium-Tygon inlet associated to the ROV temperature probe. Immediately after the recovery of the ROV, the sampling bottles were carried out to the chemical lab of the oceanographic vessel (clean lab, P 100 000; ISO8). pH measurements were performed on a subsample using a Metrohm® pH-meter with a combined pH electrode (Ingold®) for sulphide rich medium (precision ± 0.01). Measurements were made at 25°C after calibration with NBS buffers (pH 4 and 7). Sample temperatures were derived from the data recorded by the temperature probe associated with the sample inlet. The seawater samples were passed through a 0.45-µm-Millipore®-HATF filter. Dissolved copper in this paper is the operationally (by filter pore size) defined fraction <0.45 µm. A 50-mL aliquot of the filtrate was acidified at pH 2, then stored in Nalgene HDPE bottles at ambient temperature until further analysis of the total dissolved copper (TDCu). Another 100 mL aliquot,

maintained at seawater pH, was stored at -20°C in Nalgene HDPE bottles. The separation and the quantification of the hydrophobic organic copper complexes ($C_{18}Cu$), and the inorganic and hydrophilic organic complexes ($nonC_{18}Cu$) were performed on shore.

Filtrations, C_{18} extractions and analyses were carried out in class-100 laminar flow hood-equipped laboratories. Prior to use, all the items employed for sampling, filtration and storage were washed several times with diluted hydrochloric acid (pH 2, HCl suprapur®, Merck) and then rinsed with Milli-Q RG® water. The filters were soaked in diluted hydrochloric acid (pH 2) for 2 month before the cruise.

At each sampling site, in-situ measurements of total sulphide were realized using a new chemical analyzer described in Vuillemin et al. (2007 and submitted). The concentrations of dissolved oxygen and temperature were recorded using an autonomous Optode Aanderaa 3830 (Tengberg et al., 2006).

2.3 Separation and analyses of the various copper chemical species

Because conventional C_{18} silica columns are subjected to polar interactions associated with the surface free silanol groups, endcapped C_{18} columns recently developed by Interchrom® (C_{18}-S-200LRC) for solid-liquid chromatography were selected in order to obtain the non-polar organic fraction with a better accuracy. The protocol used was the one described by Mills and Quinn (1981). The C_{18} column was first washed by passing successively 10 mL of methanol (G Chromasolv®, Sigma-Aldrich), 10 mL of HCl 3.10^{-2} M (Suprapur®, Merck), 10 mL of methanol and finally 20 mL of Milli-Q water. Then, 30 mL of the sample were percolated through the column at a flow rate of 10 mL min^{-1}. Elution was achieved by passing 6 mL of a methanol/Milli Q water mixture (v/v 1:1). The resulting eluate was completed to 30 mL with acidified water at pH 2. The eluted fraction contained the $C_{18}Cu$ species, i.e. the Cu complexed with non polar hydrophobic ligands which includes colloidal Cu (Paulson et

158 al. 1994). The nonC_{18}Cu species were in the unretained fraction and consist of inorganic
159 copper (eg Cu^{2+}, $CuOH^{-}$, $CuCO_3$...) and Cu associated with some non colloidal hydrophilic
160 organic complexes.
161 Recovery tests of the endcapped columns have been conducted on a coastal seawater sample
162 (n=5). The results gave a recovery of 101±12%. 26±5 % of copper was retained on the C_{18}
163 column whereas 75±7% of copper was not retained. Column blanks were prepared by passing
164 100 mL Milli-Q® water on the C_{18} column under the same conditions as for samples. The
165 results obtained for the C_{18}Cu and the nonC_{18}Cu fractions were 0.24 ± 0.16 nM (n = 3) and
166 0.20 ± 0.11 nM (n = 3), respectively. In addition, the concentration of dissolved copper in pH
167 2-acidified Milli-Q® water was 0.17 ± 0.10 nM (n = 3). Comparison with this "reference"
168 value showed that the blank ones fell in the same range; thus, no additional contamination
169 was observed from the C_{18} extraction. Extraction reproducibility was assessed on a coastal
170 seawater sample. Under the same conditions the C_{18}Cu forms account for 26 ± 5% (n = 5) and
171 the nonC_{18}Cu forms account for 75 ± 7% (n = 5).
172 The various copper species (TDCu, C_{18}Cu and nonC_{18}Cu) were measured by stripping
173 chronopotentiometry (SCP) with a gold electrode using the standard method. This method
174 was developed in the lab (Riso et al., 1997a) and has been commonly used in estuarine and
175 coastal waters (Waeles et al., 2004 and 2005). This method is very appropriate for total
176 dissolved copper quantification in organic matter-rich seawater samples since it requires no
177 preatreatment by UV irradiation. The reproducibility is 2% and the detection limit 0.17 nM
178 (Riso et al., 1997a and 1997b). The results obtained for the analysis of copper in certified
179 seawater samples are presented in Table 1.
180
181 **3. Results and discussion**
182 *3.1 Temperature, pH and total dissolved copper*

The complete data set is presented in appendix 1. Temperature and pH levels in the 43 samples are in the range of 4.6 to 22°C and 5.4 to 7.5 respectively. These values are characteristic of the cold part of the mixing zone between the hydrothermal fluid [T = 324°C, pH 3.5 – 4.2 (Charlou et al., 2000)] and seawater (T = 4.4°C, pH 7.8). The corresponding hydrothermal input can be roughly estimated using the temperature endmember data (Von Damm et al., 1998; Charlou et al., 2000). The calculated fluid input (fluid input % = 0.314T - 1.38) is limited to 0.1 to 5.5%. The possibility of using temperature or pH as dilution tracer of hydrothermal fluid by seawater was tested using the T/pH relationship of our samples. The sigmoid trend obtained (Figure 3) can explain 86% of the variability in the data. This result confirms that at the scale and in the range studied, pH and temperature can be assumed to follow a semi-conservative process and can be used as tracers of the dilution. In this paper, pH is used preferentially as dilution tracer rather than temperature because it was measured directly in the samples whereas temperature was derived from sensor data.

The total dissolved copper concentrations (TDCu) vary between 0.03 and 5.15 µM in the samples (n = 42). These values are clearly far greater (one to three orders of magnitude) than the ones reported for deep North Atlantic waters, i.e. 1.0 to 3.2 nM (Bruland and Franks, 1983; Donat and Bruland, 1995; Saager et al., 1997; Le Gall et al., 1999), indicating a marked copper enrichment due to hydrothermal inputs. These concentrations are also lower than the 20-34 µM of total copper estimated by Von Damm et al. (1998), Charlou et al. (2000) and Douville et al. (2002) in the Tour Eiffel endmember fluid. Our copper concentrations fall within the range of the ones reported for the same area or for other hydrothermal systems colonized by vent fauna (Table 2) and they are in accordance with the ones previously reported by Geret et al. (1998) for the Tour Eiffel site. Surprisingly, the ranges are comparable to the concentrations found in other hydrothermal systems characterized by different geographical (EPR and MAR) and geological settings (Rainbow and Lucky Strike)

with likely differences in the chemical composition and the biogeochemistry of the mixing zones. However, the concentrations previously published represent the concentration found in unfiltered samples. Finally, the variability observed is important with concentrations of total dissolved copper spreading between 0.03 to 5.15 µM in a 17°C temperature and 2 pH units gradient.

3.2 Dissolved copper species concentrations

Inorganic and hydrophilic organic copper concentrations (nonC_{18}Cu) range between 0.020 and 4.85 µM whereas hydrophobic organic copper levels (C_{18}Cu) vary from 0.001 to 0.095 µM. Thus, copper on the Tour Eiffel edifice occurs mainly as inorganic or hydrophilic organic species whereas the C_{18}Cu fraction appears to be very low. This fraction, which was measured for the first time in a hydrothermal environment, accounts only for 2 ± 1% of the total dissolved copper (n = 42).

Compared to other marine environments, lower ratios of C_{18}Cu/TDCu are obtained in this hydrothermal system. As examples, in the North Atlantic and the North Pacific oceans, hydrophobic organic complexes may represent 11 to 36% of the total dissolved copper (Hanson and Quinn, 1983; Donat et al., 1986- filtration on 0.3 µm). In Mediterranean waters filtered on 0.4 µm, the C_{18}Cu/TDCu ratios were in the range of 11 to 55% (Elbaz-Poulichet et al., 1994; Yoon et al., 1999). Studies conducted in estuarine systems and coastal waters report slightly higher ratios compared to oceanic waters with values ranging from 11 to 66% (Waeles et al., 2004- filtration on 0.45 µm).

The particularly low ratios obtained for our study may ensue from two complementary causes or origins. Firstly, our experiments were conducted with endcapped C_{18} columns whereas the previously cited studies used non-endcapped ones (Hanson and Quinn, 1983; Donat et al., 1986; Elbaz-Poulichet et al., 1994; Yoon et al., 1999). The latter type of columns is

susceptible to retain the non-polar hydrophobic forms, but also a part of the polar compounds as well. The overestimation of the non-polar hydrophobic fraction by non-endcapped columns has been assessed on a coastal seawater sample prior to this study and has been also evaluated by El Sayed and Aminot (2000) on samples of Mediterranean water. From our measurements, the C_{18} fraction retained by non-endcapped columns (52 ± 7%) is twice higher that the one retained by endcapped columns (26 ± 5%). By using a Chelex-100 resin pre-treatment before the C_{18} extraction, El Sayed and Aminot (2000) found a C_{18}Cu/TDCu ratio of 6.6 ± 0.4% whereas a 3-fold ratio (21.8 ± 1.7%) was reported after extraction when using only the non-endcapped C_{18} columns.

Secondly, the low hydrophobic organic copper complexation observed in hydrothermal waters can be cross-checked with the results of Sander et al. (2007). Over their study on three different vent fields, they found that 11% of copper or more should be complexed by organic matter. By comparison, estimations in estuarine and coastal media indicated that more than 99% of copper is organically complexed (Donat et al., 1994). Organic ligands can have different origins in the hydrothermal environment studied. For example, Holms and Charlou, (2001) observed the abiotic production of linear saturated hydrocarbons (C16-C29) during the hydrothermal process in ultramafic systems. A potentially important source will be the complex detritic organic matter produced by hydrothermal faunal assemblages (Limen et al., 2001). Finally, the production of microbial exopolysaccharides have been revealed in deep sea vents (Nichols et al., 2005). These extracellular polymers have been shown to possess strong metal chelating properties (Loaec et al. 1998). Indeed, in hydrothermal systems, sulphides which form stable complexes with copper [K'(CuL) = 11.5 (Al-Farawati and van den Berg, 1999)] may be present at concentrations 250-fold higher than that of copper organic ligands. Such high sulphide levels should limit the formation of organic complexes and particularly of non-polar hydrophobic ones even though other metals such as Fe or Zn, which

have higher concentrations than Cu in hydrothermal fluids, can also compete for sulphide as potential ligands. Al-Farawati and van den Berg (1999) determined the stability constant of metal sulphides complexes in pH 8 seawater. In the presence of organic ligands, the copper organic species are dominant for sulphide < 1 nM (i.e. Cu < sulphide). At higher levels of sulphide, copper monobisulphide ($CuHS^+$) and copper dibisulphide [$Cu(HS)_2$] will constitute the major species. In this study, the total sulphide concentration was measured in situ and ranges between 0.1 to 69 µM. The resulting ratio H_2S_T/TDCu varies from 1.8 at pH 7 to 400 at pH 5.7 (H_2S_T = 40 µM, TDCu = 0.1 µM). The measured values are always in the case of a H_2S_T/Cu > 1 favouring the formation of inorganic sulphide complex (copper mono bisulphide and copper dibisulphide) rather than organic complexes according to the laboratory work done by Al-Farawati and van den Berg (1999).

3.3 Copper behaviour in the hydrothermal fluid-seawater mixing zone

Figure 4 depicts the variations of TDCu, C_{18}Cu and nonC_{18}Cu concentrations versus pH. The pH decrease indicates an increase of the hydrothermal input. TDCu levels increase from 33 to approximately 1000 nM for a pH varying between 5.6 and 6.5 and then reach a plateau (1310 ± 690 nM; n = 30) for higher pH values. The C_{18}Cu and nonC_{18}Cu forms follow the same trend than TDCu as expected from section 3.2. In the pH range of 5.6 - 6.5, these fractions increase from 1 to approximately 20 nM and from 20 to approximately 1000 nM, respectively. At pH > 6.5, their concentrations are relatively constant; i.e. 22 ± 18 nM and 1250 ± 680 nM, respectively. It is worth noting that the various copper fractions in the hydrothermal fluid-richest sampled waters that were collected in a diffuse venting area (boxed area in Figure 4) show higher levels than those observed at pH 5.6 and collected within a mussel clumps.

In this part of the mixing zone, copper does not follow a conservative behaviour since it does not show any negative correlation with pH. Theoretical copper concentrations following a simple dilution process were estimated using the data published by Charlou et al. (2000). Our data fall well above this theoretical mixing line for pH > 6, and are close or below this line for pH < 6 (Figure 4, dotted line).

In order to explain the peculiar behaviour of copper in this part of the mixing zone, the fluctuations of dissolved oxygen (O_2) and total sulphide (ΣS) in the studied area are also presented (Figure 5 and 6). Dissolved oxygen was measured in situ with an Aanderaa optode moored in a neighbouring mussel clump. The results obtained show an expected decrease in oxygen concentration when increasing the hydrothermal input of reduced species. Total sulphide was measured in situ at the 12 sampling points with the chemical analyzer CHEMINI. The total sulphide vs. pH curve presents an inflexion point between pH 6.5 and 7. The data clearly highlight the presence of a transition area at a pH around 6.7 between high sulphide/low oxygen waters and low sulphide/high oxygen waters. It can be seen that the lowest Cu concentrations measured at pH < 6 (Figure 4) correspond to high sulphide/low oxygen area. In this acidic part of the mixing zone, the copper concentration might be controlled by precipitation with sulphide. As a matter of fact, James and Elderfield (1996) observed in the Snake pit vent field (MAR) that only 43% of copper was present in the dissolved fraction (< 0.4 µM). Copper occurs mainly as sulphides (such as chalcopyrite) that form large sized grains and settle rapidly in the near field region (Trocine and Trefy, 1988; Feely et al., 1994).

For pH > 6, the dissolved Cu concentrations follow a trend opposite to a conservative behaviour: TDCu concentrations increase to reach a plateau at pH around 6.5. In the same pH range, total sulphide levels are characterized by a sharp drop from 40 to ca. 10 µM. In the pH range 6 - 6.5, the copper enrichment observed could be related to the dissolution of settled

copper sulphide particles in the presence of dissolved oxygen. The speciation of dissolved copper obtained in this study supports this hypothesis as most of the dissolved copper (> 96%) is present under inorganic or hydrophilic organic complexes. Such an oxidative redissolution (oxic alteration) phenomenon has already been advanced to explain the variations of dissolved copper in hydrothermal fluids during the oxidation of chalcopyrite by seawater or diluted fluids (Metz and Trefry, 2000). In hydrothermal sediments from the EPR, Cu is associated to Fe oxyhydroxides that can undergo dissolution under oxic conditions and finally precipitate as goethite (Dunk and Mills, 2006). In the sediments of the TAG hydrothermal field (26°N, MAR), Severmann et al. (2006) reported elevated dissolved Cu concentrations (< 0.2 µm) that might be caused by reaction between hydrothermal minerals with oxygenated seawater within the suboxic/oxic area and producing a remobilization of Cu. Oxidative redissolution processes were also observed in other marine systems. For example, Paucot and Wollast (1997) reported in the Scheldt estuary a removal of dissolved Cu (< 0.45 µm) at low salinities due to sulphide precipitation in the anoxic part of the system. At higher salinities and higher oxygen content, an increase of the dissolved fraction occurred as the result of the redissolution of sulphide in oxic conditions.

For pH values higher than 7.5, one should expect in our system a strong decrease of dissolved copper to reach the typical concentrations values of deep seawater (i.e. in the range 1-3 nM).

In Figure 7, we propose a conceptual model forecasting the copper behaviour over the entire hydrothermal fluid-seawater mixing zone. Three major phenomenon should be considered: (i) the precipitation of copper with hydrogen sulphide in the anoxic area close to the vent orifice at pH < 6; (ii) the oxidative redissolution of accumulated copper sulphides particles to form inorganic and hydrophilic organic complexes with increasing oxygen concentrations for pH between 6 and 7.5 and (iii) the dilution of high metal content waters with low Cu seawater.

3.4 Ecological significance

The observed remobilisation of dissolved copper in the part of the mixing zone that corresponds to the preferred habitat of *Bathymodiolus azoricus* faunal assemblages can have an important biological significance. This mytilid mussel is the dominant “engineering” species colonizing the Tour Eiffel edifice (Desbruyères et al., 2001). This species hosts a dual symbiotic population in its gills composed of methanotroph and sulfoxidant microorganisms (Fiala-Medioni et al., 2002; Duperron et al., 2006). The morphology of its intestine suggests also a residual dependence on filter feeding (Fiala-Medioni et al., 2002). Metals like Fe, Zn and Cu are essential for live organisms but may become toxic if present in excess. Bioaccumulation in *B. azoricus* from the MAR occurs mainly in the gills that correspond to the main interface between the organism and its environment and also in the digestive gland (Cosson et al., 2008). Furthermore, metal bioaccumulation appears to be related to the specificity of each vent field and reflects partly the chemical composition of the hydrothermal fluid (Cosson et al., 2008). Even though the main bioaccumulation pathway of metals in bivalves comes from their trophic uptake (Wang, 2002; Wang and Rainbow, 2005), it was shown that they can directly intake the metals from the solute phase through permeable surfaces including the gills (Marsden and Rainbow, 2004). This pathway will be strongly influenced by the speciation of dissolved metals, including the hydrophilic / hydrophobic properties of metallic complexes. Metals in solution may be taken up across permeable membranes via different transport routes. A metal ion can either bind with a carrier protein that mediate the transport through the membrane or it can cross the membrane using ionic channels formed by a protein with a hydrophilic core. In addition, some hydrophobic complexes can cross directly the bilayer lipidic cell membrane and can be released in the cytoplasm. Nevertheless, this entry route appears to be quite limited (Simkiss, 1998). In summary, the main intake processes of dissolved metals involve ionic channels and are

directly linked to the concentration of labile metal species (Simkiss, 1998). Therefore, the presence of a secondary source of dissolved copper, associated with the accumulation of metal sulphide particles, could play a significant role in the distribution of fauna in the different habitats available at vents. Whether different hydrothermal fauna have different tolerance and/or adaptation to deal with copper concentrations remains to be investigated.

4. Conclusion

In this study focused on the Tour Eiffel edifice (Lucky Strike vent field, MAR), levels of total dissolved copper range from 0.03 µM to 5.15 µM. These values are much higher than the ones reported for deep-sea oceanic waters but are in accordance with the ones previously obtained in this area. Our data from speciation measurements show, for the first time, that the hydrophobic organic fraction of copper ($C_{18}Cu$) is very low (2 ± 1%) in the hydrothermal fluid-seawater mixing zone. On the other hand, copper is found mainly as inorganic and hydrophilic organic complexes (non$C_{18}Cu$). This result is especially interesting because this latter fraction is liable to be more bioavailable than the former one. The distribution of copper along the pH gradient shows the same pattern of evolution for each fraction. These fractions highly increase from pH 5.6 to pH 6.5, then remain relatively constant at pH > 6.5. Oxygen and sulphides data demonstrate that the copper anomaly at pH 6.5 is found in the transition area between suboxic and oxic waters. Thus, the increase of dissolved copper should correspond to the oxidative redissolution of copper sulphide particles formed in the vicinity of the fluid exit. Moreover, our data indicate that the inorganic and hydrophilic organic fraction is the one to be mainly affected by this oxidative redissolution mechanism.

Further work will be done on the whole mixing zone from the hot or diffuse emission to the seawater pole to check the proposed behaviour. In addition, the data acquired during this

study will be used to look at the relationship between chemical gradients and species distribution within *Bathymodiolus azoricus* mussel assemblages,

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Figure
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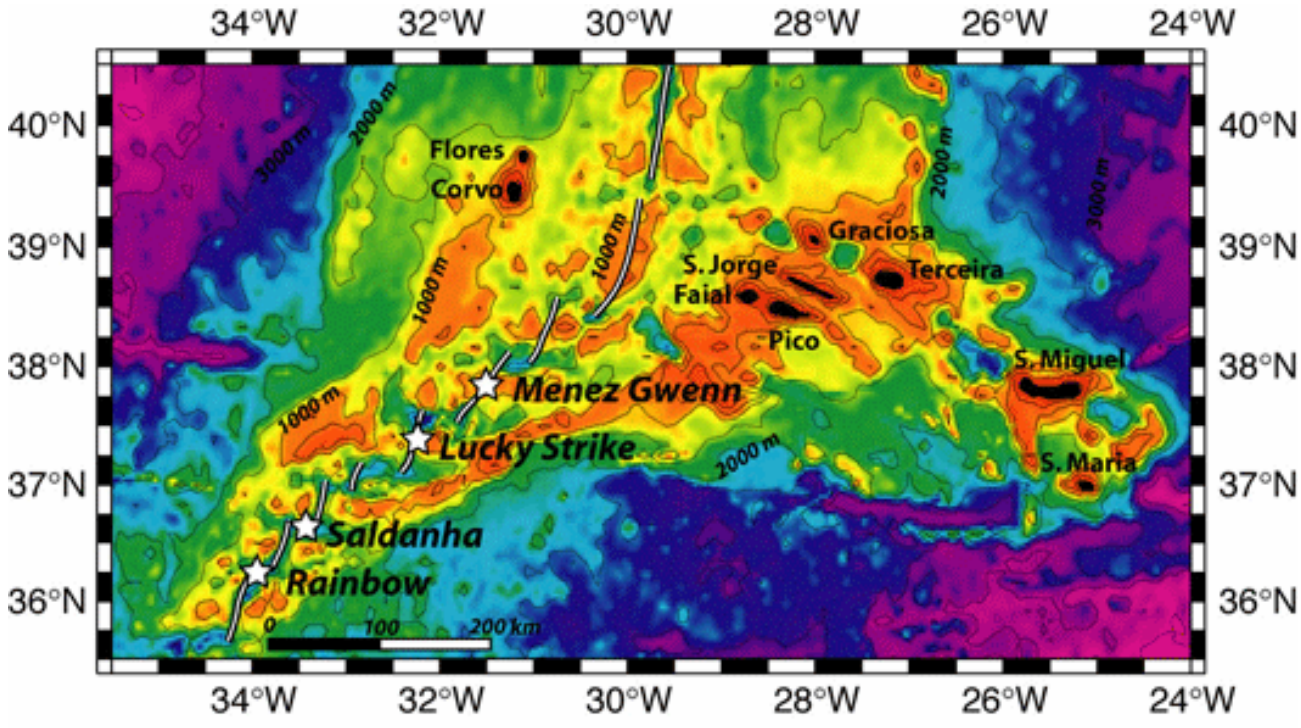

Fig. 1- Simplified bathymetric map of the Mid-Atlantic Ridge near the Azores (seafloor depths in meters), showing the location of the four main hydrothermal vent fields. (http://www.momarfr.org/)

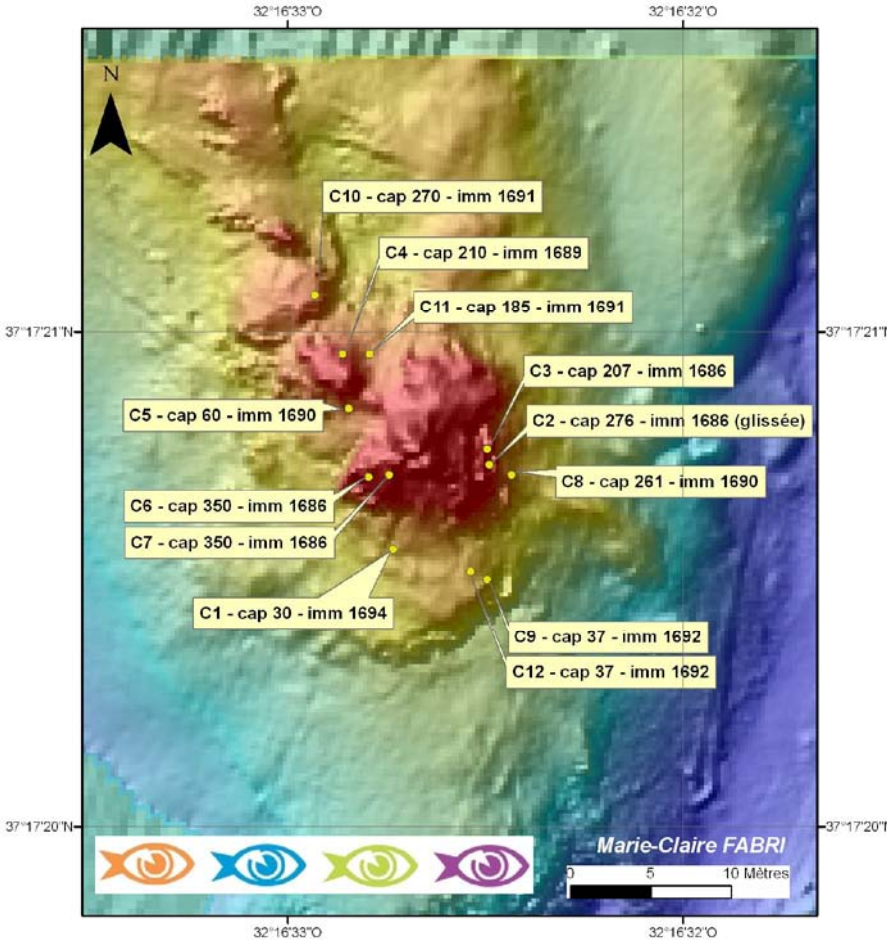

Fig. 2- Bathymetric map of the Tour Eiffel active edifice and location of the 12 sampling stations C1 to C12 (Sarrazin et al., 2006).

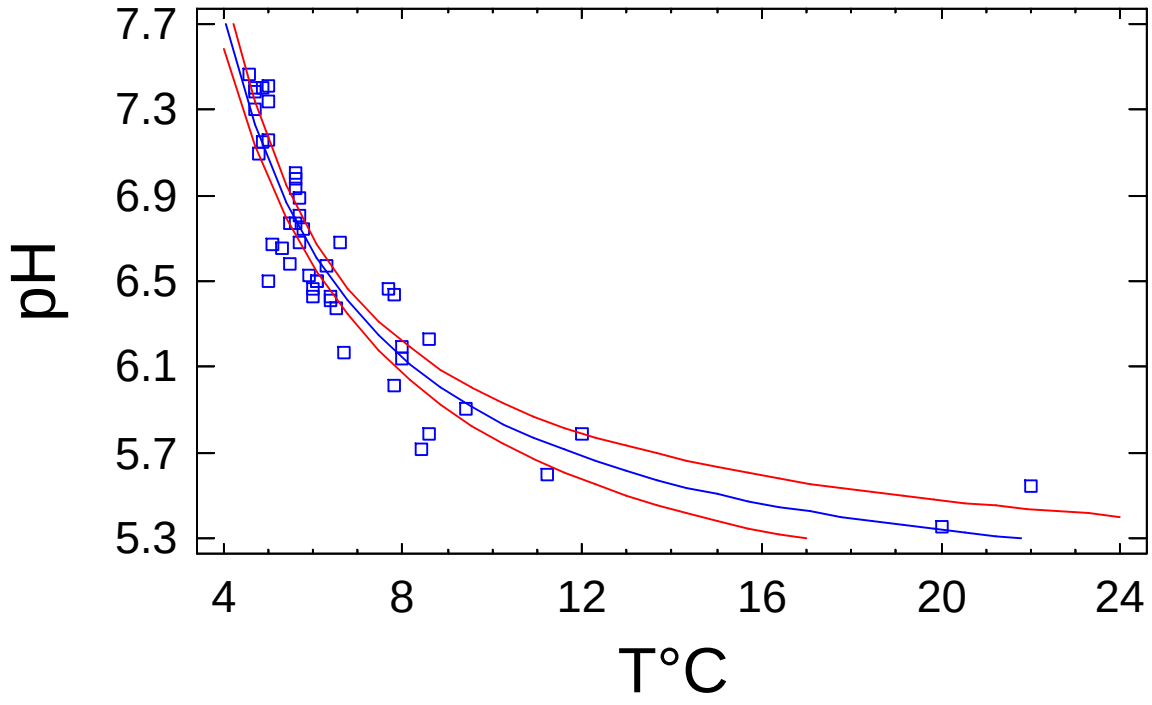

Fig. 3- Temperature/pH relationship obtained in the cold part of the mixing zone. $pH=\exp^{(1.582+1.857/T)}$, R^2 = 86.6%, n = 42. The external lines represent the confidence interval at 95% (Statgraphics Plus).

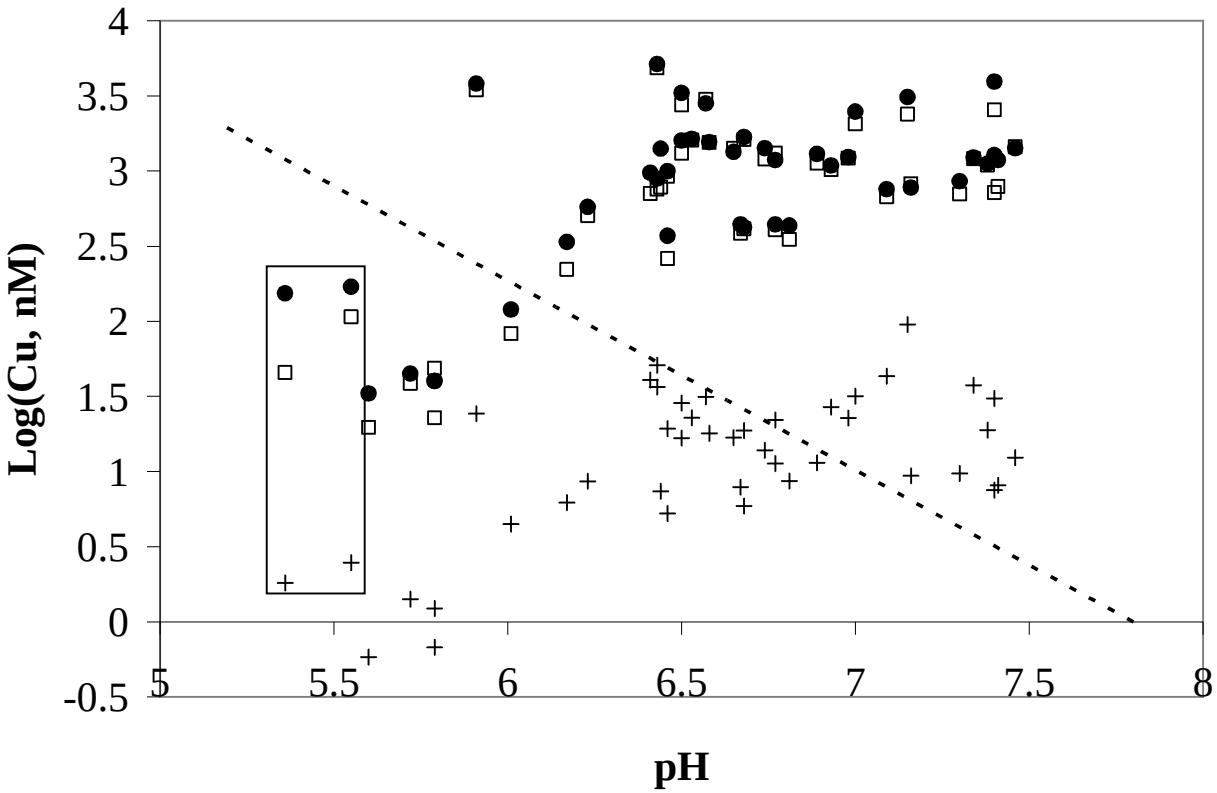

Fig. 4- Distribution of copper concentrations [Log (Cu, nM)] vs pH. TDCu (•), C_{18}Cu (+) nonC_{18}Cu (□). The boxed area indicates the samples with the lowest pH/highest temperature (~ 22°C) collected in a diffuse venting area. The dotted line represents a theoretical dilution of hydrothermal copper [pH = 4.3, Cu = 26000 nM, (Charlou et al., 2000)] by seawater, pH 7.8, Cu = 1 nM.

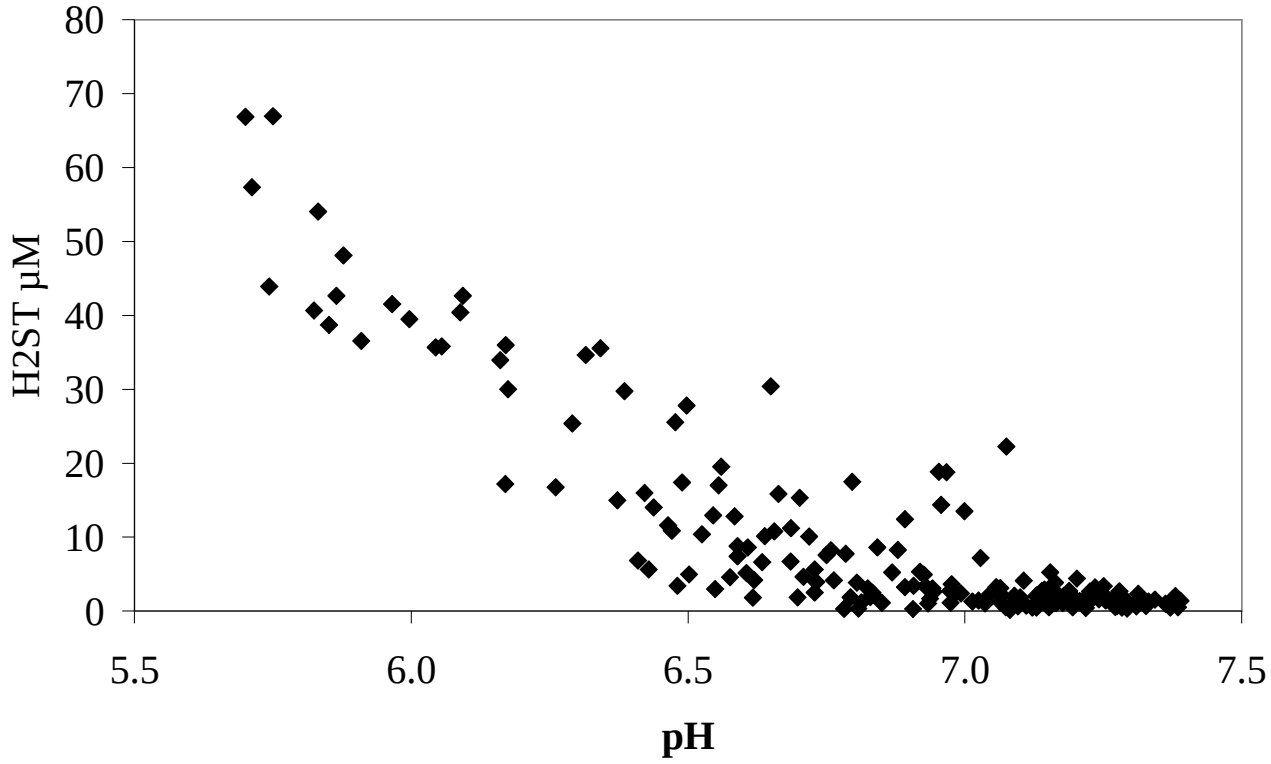

Fig. 5- Concentration of total sulphide measured in situ with the CHEMINI chemical analyzer on the 12 sampling points (T = 4.5 to 11.7°C, n = 189).

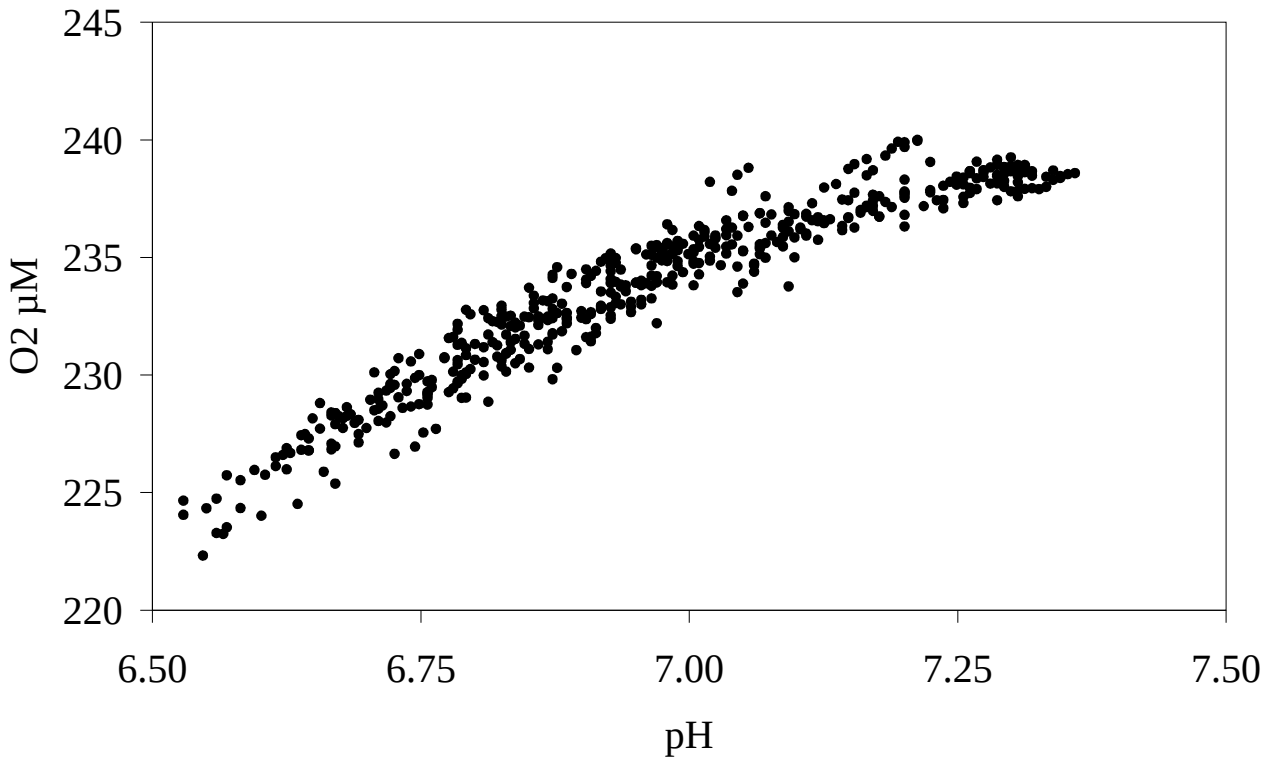

Fig. 6- Concentration of dissolved oxygen measured in situ by an Aanderaa optode in a neighbouring mussel clump during 4 hours (2 mes / min, n= 480, T = 4.5 to 6.3°C).

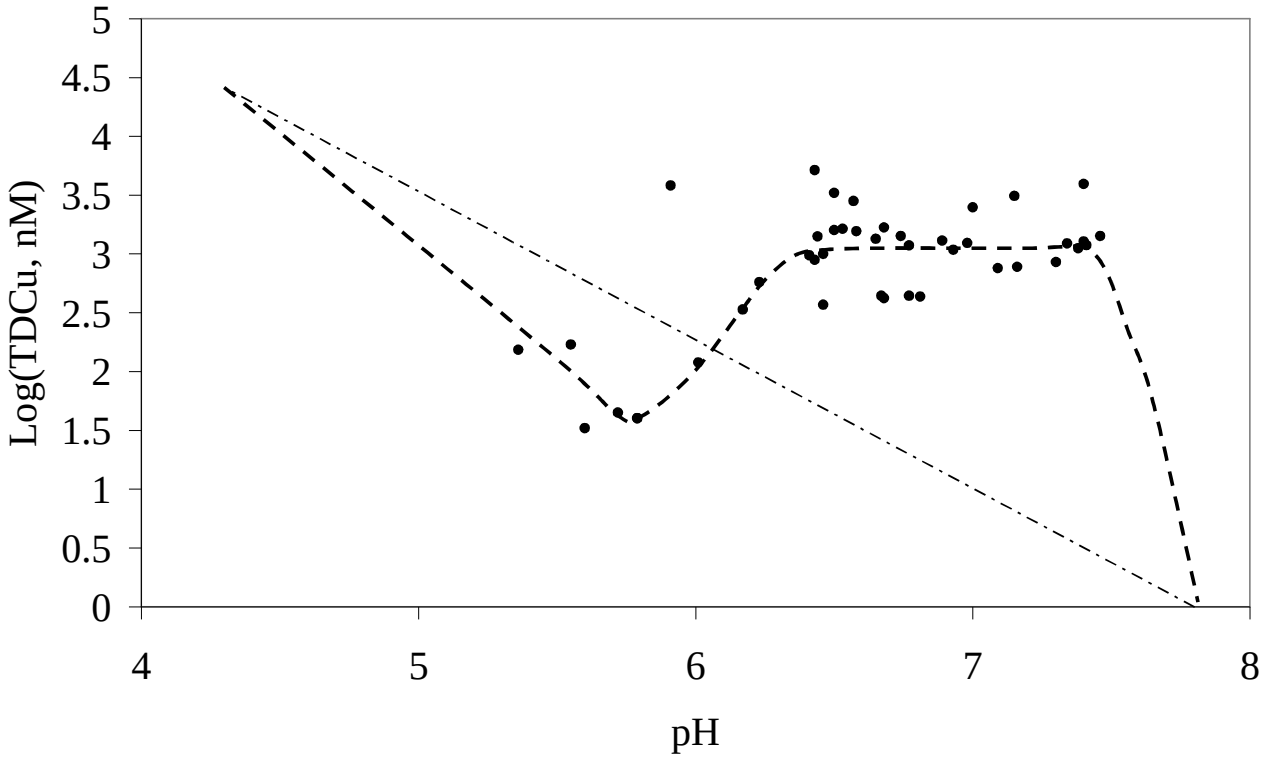

Fig. 7- Proposed schematic behaviour of dissolved copper in the hydrothermal fluid-seawater mixing zone. The dotted straight line represents the theoretical dilution of hydrothermal copper (pH = 4.3, T = 325, Cu = 26000 nM) by seawater.

TABLES

	Cu (nM)	
	Certified value	Measured value
NASS-5	4.7 ± 0.7	4.4 ± 0.2
CASS-3	8.1 ± 1.0	7.6 ± 0.5
SLEW-2	25.7 ± 1.7	27.2 ± 1.4

Table 1- Analysis of Cu in certified seawater samples: NASS-5 oceanic seawater, CASS-3 coastal seawater, SLEW-2 estuarine water. Values are expressed as mean ± confidence interval (95%)

Hydrothermal fields	Cu (µM)	T°C	pH	References
EPR, 9°N	0.08 - 2.10 ***	13 - 176	5.3 - 6.4	Di Meo-Savoie et al., 2004
EPR, 13°N	1.38 - 3.27*	-	5.7 - 7.5	Desbruyères et al., 1998
EPR, 13°N	0.18 - 1.6*	3.8 - 20	-	Sarradin et al., 2008
MAR, Rainbow	0.14 - 3.20*	4.7 - 25	6.3 - 7.5	Geret et al., 2002
MAR, LS, Tour Eiffel	0.02 - 2.05*	-	-	Geret et al., 1998
MAR, LS	1.13 **	4.3	6.2	Kadar et al., 2005
MAR, LS, Tour Eiffel	**0.03 - 5.15 ****	**4.6 - 22**	**5.4 - 7.5**	**This study**

Table 2- Concentrations of copper in various hydrothermal systems colonized by hydrothermal fauna. MAR: Mid Atlantic Ridge; EPR: East Pacific Rise; LS: Lucky Strike vent field. * unfiltered samples, ** samples filtered on a 0.45 µm filter. *** samples filtered and centrifugated.

Appendix 1 : pH, temperature and total dissolved copper obtained in the 43 samples.

pH measurements were performed onboard using a Metrohm® pH-meter with a combined pH electrode for sulphide rich medium. Sample temperatures were derived from the data recorded by the temperature probe associated with the sample inlet. Samples were filtered on board through a 0.45-µm-Millipore®-HATF filter. The separation and the quantification of the hydrophobic organic copper complexes ($C_{18}Cu$), and the inorganic and hydrophilic organic complexes ($nonC_{18}Cu$) were performed on shore.

Sample	pH	T°C	TDCu (µM)	$nonC_{18}Cu$ (µM)	$C_{18}Cu$ (µM)
296 A2	5.6	11.2	0.033	0.02	0.001
300 E1	5.72	8.4	0.045	0.04	0.001
300 D3	5.79	8.6	0.040	0.05	0.001
301 C1	5.79	12	0.040	0.02	0.001
300 D2	6.01	7.8	0.120	0.08	0.004
296 A1	6.17	6.7	0.337	0.22	0.006
297 A1	6.23	8.6	0.576	0.51	0.009
300 B2	6.41	6.4	0.976	0.71	0.041
296 B1	6.43	6	0.890	0.76	0.036
296 B2	6.43	6.4	5.151	4.85	0.051
301 C2	6.44	7.8	1.410	0.78	0.007
297 C1	6.46	7.7	1.00	0.92	0.019
300 B3	6.46	6	0.37	0.26	0.005
297 B2	6.5	5	3.31	2.76	0.029
300 C3	6.5	6.1	1.60	1.31	0.017
300 C1	6.53	5.9	1.64	1.61	0.023
301 B2	6.57	6.3	2.82	3.00	0.031
300 C2	6.58	5.5	1.56	1.55	0.018
301 B1	6.65	5.3	1.34	1.42	0.017
296 A3	6.67	5.11	0.44	0.39	0.008
297 B3	6.68	5.7	0.42	0.41	0.006
301 B3	6.68	6.6	1.68	1.62	0.019
301 A2	6.74	5.8	1.42	1.21	0.014
300 D1	6.77	5.6	1.18	1.31	0.022
300 B1	6.77	5.5	0.44	0.41	0.011
300 A3	6.81	5.7	0.43	0.35	0.009
297 B1	6.89	5.7	1.30	1.13	0.011
300 A1	6.93	5.6	1.09	1.02	0.027

300 A2	6.98	5.6	1.24	1.22	0.023
301 A1	7	5.6	2.49	2.06	0.032
297 E2	7.09	4.8	0.76	0.67	0.043
297 C3	7.15	4.9	3.11	2.40	0.095
301 A3	7.16	5	0.78	0.82	0.009
301 C3	7.3	4.7	0.86	0.70	0.010
297 D2	7.34	5	1.23	1.21	0.038
301 D1	7.38	4.7	1.12	1.10	0.019
297 D1	7.4	4.7	3.95	2.56	0.031
301 D2	7.4	4.9	1.28	0.72	0.008
301 D3	7.41	5	1.19	0.79	0.008
297 D3	7.46	4.6	1.42	1.45	0.012
297 C2	5.91	9.4	3.82	3.47	0.024
296 C1	5.55	22	0.17	0.11	0.002
296 B3	5.36	20	0.15	0.05	0.002