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Relationship between the occurrence of filamentous bacteria on *Bathymodiolus azoricus* shell and the physiological and toxicological status of the vent mussel

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Abstract:

The edifice walls of the Eiffel Tower hydrothermal vent site (Mid-Atlantic Ridge, Lucky Strike vent field) are populated with dense communities of dual symbioses harboring vent mussel *Bathymodiolus azoricus*, some of which are covered by white filamentous mats belonging to sulfur-oxidizing bacteria. Mussels were collected in both the presence and absence of the filamentous bacteria. A sample of the filamentous bacteria was collected and water measurements of temperature, CH_4 and H_2S were recorded at the collection area. The whole soft tissues were analyzed for total lipid, carbohydrate and total protein. Metallothioneins and metals (Cu, Fe and Zn) levels were determined in the major organs. The results showed no significant physiological and toxicological evidence that emphasizes the influence of associated sulfur-oxidizing filamentous bacteria on *B. azoricus* mussel shells. However, *B. azoricus* mussel seems to be well adapted to the assorted physico-chemical characteristics from the surrounding environment since it is able to manage the constant fluctuation of physico-chemical compounds.

Keywords: Biomarkers; Eiffel Tower; Filamentous bacteria; Metallothioneins; Metals; Vent mussel

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1. Introduction

Lucky Strike is one of the largest known active vent fields (37° 18′ N, 32° 16′ W), located in the Mid-Atlantic Ridge between 1730 and 1736 m depth. The hydrothermal fluid, emitted at temperature ranging between 170 and 324 °C, presents characteristics (temperature, chlorinity, gas and metal concentration) that vary from site to site within the field (Charlou et al., 2000 J.L. Charlou, J.P. Donval, E. Douville, P. Jean-Baptiste, J. Radford-Knoery, Y. Fouquet, A. Dapoigny and M. Stievenard, Compared geochemical signatures and the evolution of Menez Gwen (37°50′N) and Lucky Strike (37°17′N) hydrothermal fluids, south of the Azores triple junction on the Mid-Atlantic Ridge, Chem. Geol. 171 (2000), pp. 49–75. Article | PDF (602 K) | View Record in Scopus | Cited By in Scopus (71)Charlou et al., 2000). The hydrothermal vent area is distributed around a lava lake, bound by the summits of three volcanic cones (Radford-Knoery et al., 1998). Both well-defined active chimneys such as Eiffel Tower belching out very hot fluids and zones where hydrothermal activity is more diffuse, can be found at Lucky Strike (Desbruyères et al., 2001). Eiffel Tower, located at 200 m from the southeastern edge of the circular lava lake, consists of chimneys combined into an edifice rising 7 m above the neighboring seafloor (Radford-Knoery et al., 1998). The structure presents numerous high temperature smokers and cracks emitting lower temperature fluids. It is colonized nearly uniformly by the vent mussel Bathymodiolus azoricus, several patches of mussel been covered by dense white filamentous microbial mats (Sarradin et al., 1999; Desbruyères et al., 2001) while other are not at all. The filamentous microbial mats are mainly composed of a species of *Beggiatoa* (Mattison et al., 1998). This species is a colonizer, very abundant in environments characterized by the presence of hydrogen sulfide (Wörner and Zimmermann-Timm, 2000). These chemoautotrophic bacteria gain energy by the oxidation of reduced sulfur compounds (Nelson et al., 1989; Hagen and Nelson, 1997; Erbacher and Nelskamp, 2006). *B. azoricus* hosts ‘‘dual symbioses’’, involving the stable coexistence of chemoautotrophic (also referred to as thiotrophic) and methanotrophic bacteria harbored within the gill (Fiala-Médioni et al., 2002; Duperron et al., 2005). Dual symbioses provide obvious advantages to host individuals recruiting to environments where the availability of substrates is unpredictable or fluctuating (Cavanaugh et al., 1992; Fiala-Médioni et al., 2002). This dual symbioses has also been described in others mussel species that live in reducing environments with high sulfide and methane concentrations, such as hydrothermal vents (Duperron et al., 2005; Stewart et al., 2005) and cold seeps (Fisher et al., 1993). The physiology and biochemistry of the hydrothermal vent mussel *B. azoricus* must be adapted to the rapid fluctuating composition of its environment composed of a mixture of seawater and hydrothermal fluid (Childress and Fisher, 1992). The presence and absence of sulfur-oxidizing filamentous bacteria on the hydrothermal mussel beds could be an indicator of a fluctuating environment, since these chemolithoautotrophic bacteria proliferate in environments with reduced sulfur compounds (Brinkhoff and Muyzer, 1997) and grow in habitats with oxic-anoxic interfaces (Moyer et al., 1995). These chemical conditions may alter the chemistry of mussel habitats (LeBris et al., 2006) and consequently their physiological and toxicological condition. The aim of our investigation was to study the physiological condition and metal accumulation of *B. azoricus* collected from a mussel bed where the presence and absence of filamentous bacteria were observed.

2. Material and Methods

2.1. Sampling

Samples were collected during the EXOMAR cruise (with the R/V "Atalante") in July 2005, at 1690 m depth on the Eiffel Tower hydrothermal site. The mussels were sampled by the manipulator arm and brought to the surface using the Remotely Operated Vehicle (ROV) "*Victor 6000*". Samples were collected in two neighboring areas, where mussel shells were covered by filamentous bacteria (designated hereafter as "mussels + mats") or not covered (designated hereafter as "mussels"). From each area 25 individuals were collected and measured. Individuals from the group "mussels + mats" presented a mean total length of 4.3 cm (± 0.5 SD) and individuals from the group "mussels" presented a mean total length of 3.0 cm (± 0.3 SD). For each group, the whole soft tissues of 15 mussels were separated from the shells and kept frozen (-80ºC) until lyophilization and analysis. Left 10 mussels were dissected into gill, mantle, foot, digestive gland and remaining soft tissues and kept frozen (-80ºC) until lyophilization and analysis. A sample of microbial mats, that covered the mussels, was collected using the water pumping device of the ROV "VICTOR 6000". Water samples (5 litres) were filtered on board and filters were preserved at -80ºC for further analysis.

2.2. Lipid analysis

Because of small soft tissue weights, 5 mussels were pooled together for single measurement of total lipids levels at each group. The lipid content was determined according to the modified method of Bligh and Dyer (1959) by extracting lipids from a dry powdered in a water-dichloromethane–methanol mixture and by weighing after evaporation to dryness, the organic layer. Level was expressed as mg g^{-1} of dry weight.

2.3. Carbohydrate analysis

The whole soft tissue of 5 individuals from each group of mussels was used for carbohydrate analysis. The carbohydrate content was determined colorimetrically in a NaCl extract, in the presence of 5% phenol and concentrated H_2SO_4, as described by Dubois et al. (1956). The concentration was determined in glucose equivalents from a glucose calibration curve using glucose as a standard. The levels of carbohydrate were expressed as mg g^{-1} of dry weight.

2.4. Samples preparation for total protein, metallothionein and metal analyses

The tissues of the 10 dissected mussels from each group, were lyophilized, weighed and homogenized in 6 ml of ice-cold 100 mM Tris buffer, pH 8.1, containing 10 mM ß-mercaptoethanol. The homogenates were centrifuged for 30 min at 25 000 g, at 4°C to separate the supernatants (S_1) from the insoluble fraction (pellets), used for the study of intracellular metal distribution. Aliquots (1 ml) of the S_1 were used for metallothioneins determination. Pellets and remaining supernatants were digested in an Ethos Plus microwave oven with 5 ml of HNO_3 (69% v/v) for metal analysis.

2.4.1. Total protein analysis

Total protein levels were determined in supernatants S_1 from whole soft tissues of 5 individuals from each group of mussels, following the BioRad protein assay kit for the Bradford method (Bradford, 1976). BSA (Bovine Serum Albumin) was used as reference standard. Results were expressed as mg g^{-1} of dry weight.

2.4.2 Metallothionein and metal analysis

The aliquot of the supernatant S_1, was heat-denatured (90°C, 15 min) and centrifuged (13 000 g, 10 min, at 4ºC) in order to separate the thermostable metallothioneins (MTs) from thermolabile proteins. The heat stable fractions (S_2) were used for quantification of MTs by Differential Pulse Polarography (DPP) according to Olafson and Sim (1979) improved by Thompson and Cosson (1984). A standard addition calibration curve was obtained using rabbit liver MT-I as reference. Results were expressed as mg g^{-1} of dry weight.

After pellets and remaining supernatant S_1 digestion, solutions were dried at 60ºC and diluted by adding 2 ml 0.5N HNO_3. Metal levels (Cu, Fe, Zn) were measured by flame atomic absorption spectrophotometry (AAS) with deuterium background correction**.** The accuracy and precision of the method used were established by regular analysis of certified reference materials of mussel tissue CE278 (European Reference Materials of Belgium) and lobster hepatopancreas TORT-2 (National Research Council of Canada) (Table 1). Certified reference materials and blanks were taken through the procedure in the same way as the samples. Metal levels were calculated and expressed as mg g^{-1} of dry weight.

2.5. Microbial mat molecular diversity

A preliminary study of the microbial mat diversity was performed by using 16S rRNA gene sequencing. DNA was extracted from frozen mat sample pellets as described in Alain et al. (2002). Archaeal DNA was amplified using the primer A24F (5’-TTC CGG TTG ATC CTG CCG GA-3’) and the reverse primer 1407R (5'-GAC GGG CGG TGW GTR CAA-3'). Bacterial DNA was amplified using the primer E8F (5’-AGA GTT TGA TCA TGG CTC AG-3’) and the reverse primer U1492R (5’-GTT ACC TTG TTA CGA CTT-3’). PCR reactions were performed on a Robocycler Gradient 96 (Stratagene) (Wery et al., 2002; Nercessian et al., 2003). PCR products were then checked on a 0.8% (w/v) agarose gel and directly cloned using the TOPO TA Cloning® kit (pCR2.1 vector), according to the manufacturer's instructions (Invitrogen). Sequences were analyzed as previously described by Postec et al. (2005).

2.6. *In situ* temperature measurements, CH_4 and H_2S estimated values

Eight autonomous temperature probes (thermistor, Vemco Minilog 12 TR 64K probes) were deployed on each sampling point for two days. The temperature data were corrected against the bottom seawater temperature (4.4°C). The sampling period was 30 seconds. CH_4 concentrations were estimated using the significant Temperature/CH_4 linear relationship obtained during the ATOS cruise on 16 samples from the Eiffel Tower edifice (Sarradin et al., 2003). Total sulfide (ΣS) concentrations were estimated using the significant Temperature/ΣS linear relationship obtained in 2006 during the MoMARETO cruise with the CHEMINI in situ chemical analyzer (Vuillemin et al., 2009). Results were expressed as µmol l^{-1}.

2.7. Statistical analysis

All the results are given as mean level by individual/tissues dry weight. The statistical calculations were performed with STATISTICA software (6.0 release, StatSoft). Data were checked for normal distribution and homogeneity of variance (Leven's test). Non-parametric tests (Kruskall–Wallis and Mann–Whitney) were performed when data were not normally distributed or when they exhibited heterogeneous variances.

3. Results

3.1. Biochemical composition

Lipid, carbohydrate and total protein results per group of mussels, are shown in Table 2. No significant difference was found between the two groups of mussels (Mann-Whitney, $p>0.05$) for lipid, carbohydrate and total protein levels.

3.2. Preliminary microbial mat diversity study

Phylogenetic analysis of bacterial clone library evidenced a very large diversity of uncultured bacteria within the α,δ,γ and ε-proteobacteria. Bacteria belonging to Cytophaga/Flavobaceria/bacteroides group, planctonomycetes and actinombacteria were detected. Within the γ-proteobacteria, sequences belonging to order Thiotricales were identified. The order Thiotricales includes the filamentous sulfur-oxidizing bacteria such as *Beggiatoa*, *Thioploca* and *Thiotrix*. The archaeal diversity appeared to be very low. All the sequences were located in the marine Crenarcheaota group I and dominated by one phylum closely related to the newly described (and first cultivated species within this group) ammonia-oxidizing Crenarchaeota "*Nitrosopumilus maritimus*" (Konneke et al., 2005). Microscopic observation showed the presence of large intracellular vacuoles surrounded with sulfur granules in some large bacterial filaments, described earlier in many of sulfur-oxidizing species (Godfroy, personal observation).

3.3. *In situ* temperature measurements, CH_4 and H_2S estimated values

Table 3 presents the results of the temperature measured, CH_4 and H_2S estimated values at the environment surrounding the two groups of mussels studied. The temperatures measured within the two groups presented significant differences (Mann-Whitney, $p<0.05$). CH_4 and H_2S estimated values are distinct between the two groups of mussels. The temperature measured as well as both estimated values of CH_4 and H_2S presented higher mean in the group "mussels + mat" than "mussels" group. The temperature standard deviation, minimum, maximum and range were also higher in the environment characterized by the presence of microbial mats. The increase of relative temperature, compared to ambient seawater temperature (4.4°C), was +0.2°C within the "mussels" group and up to +3°C within the "mussels + mat" group. The temperature variations sustained by the "mussels" group were less extensive than those faced by "mussels + mat" group. Assuming that temperature can be used as a semi conservative tracer of the vent fluid dilution (Johnston et al., 1988; LeBris et al., 2006), it is possible to estimate the hydrothermal input in the studied environment using the ambient seawater (4.4°C) and the undiluted hydrothermal fluid (324°C) temperatures (Charlou et al., 2000). Consequently, the input of hydrothermal fluid observed in the sampled sites ranged between 0.2% at a temperature of 4.9°C to 1% at a temperature of 7.4°C.

3.4. Metallothionein and metal levels

Table 4 shows the levels of metals and metallothionein (MT) in the different tissues of both mussel groups. Results are presented by tissue analyzed and by group of mussels. Metal in the vent mussel can be ranked in the following order according to the levels found: Fe>Zn>Cu, for individuals from the group "mussels" and Zn>Fe>Cu, for individuals from the group "mussels + mat". Only at group “mussels” were found significant differences (Kruskal-Wallis, $p<0.05$) between the metal levels**.** The digestive gland was the organ which presented the highest levels of metals and MT. In this organ Fe levels were found statistically different between groups (Kruskal-Wallis, $p<0.05$), with higher levels found at “mussels” group. No significant difference (Kruskal-Wallis, $p>0.05$) was found between the distribution (soluble/insoluble fractions) of metals within the analyzed tissues. Metallothionein levels in the digestive gland are not statistically different between groups (Mann-Whitney, p>0.05).

4. Discussion

4.1. Physiological status

The dual endosymbiosis involving sulfur and methane oxidizers is one of the mechanisms developed by *B. azoricus* to use the chemical energy of hydrothermal environment. There is some empirical evidence that the relative abundance of dual symbiosis in this species can vary in response to environmental parameters (Fiala-Médioni et al., 2002; Duperron et al., 2005). Moreover, studies carried out in Eiffel Tower site demonstrate a higher concentration of sulfide (2.1 mM) than methane (0.68 mM) in end-member fluids (Charlou et al., 2000) and accordingly, a dominance of chemoautotrophic symbionts in the gills of *B. azoricus* from this site (Trask and Van Dover, 1999; Salerno et al., 2005; Duperron et al., 2006). The estimated values of CH_4 and H_2S presented in this study lead us to consider that each group of mussels has different energy sources. The site where "mussels + mats" were collected, presented higher H_2S values than the site "mussels" are living at. H_2S contributes to the proliferation of these sulfur-oxidizing filamentous bacteria that uses the sulfide and the oxygen available (Wörner and Zimmermann-Timm, 2000) in their proximal environment. The symbiotic sulfur-oxidizing bacteria use the energy produced by the oxidation of reduced compounds, such as sulfide, as energy source to produce organic compounds, acting as primary producers for their host (Fiala-Médioni et al., 2002). Therefore, it was suggested the possibility of the sulfur-oxidizing filamentous bacteria consume part of the environment sulfide also needed by sulfur-oxidizing symbionts. The consumption of sulfide by free-living filamentous bacteria and consequently depletion of this compound available for chemoautotrophic symbionts utilization may influence the physiological status of the mussels associated with filamentous bacteria. Several biochemical markers, such as lipids, carbohydrates and total proteins, were use to study the possible differences on physiological condition between the group "mussels + mats" and the group "mussels". The analysis of such biochemical parameters in hydrothermal vent animals has proven to be a rewarding approach to understand their biology (Childress and Fisher, 1992). Furthermore, lipids are an important source of energy that can be used during periods of food shortage and as an energy reserve for the successful larval development (Fraser, 1989). Mussels use carbohydrates as energy reserves that are metabolised into lipids during egg maturation (Kopp et al., 2005). Proteins are of fundamental importance in mussels as they are used in several biological functions for maintenance, growth and reproduction (Olsson et al., 2004). Therefore, the variation of these biochemical parameters could be considered a valuable indicator of organism physiological condition (Lagadic et al., 1997). The "mussels + mats" group and "mussels" group did not show differences between the amounts of lipids, carbohydrates and total proteins. Consequently we can not put forward differences between their physiological conditions by this mean. The preliminary study made in the microbial mat collected, identified the presence of several groups of free-living bacteria including the sulfur-oxidizing filamentous type. However it is unknown, for now, the relative abundance of these bacteria at the microbial mat. The similar physiological status found between the two groups of *B. azoricus* mussels studied may indicate a low relative abundance of sulfur-oxidizing filamentous bacteria and consequently low competition for environmental sulfide. From the temperature measurements made in both collection areas, we can assume that the environment surrounding the studied mussels is situated in the really cold part of the mixing zone between the ambient seawater and the hydrothermal fluid (Sarradin, personal observation). However, the “mussels + mats” group presented relatively high temperatures, high levels of ΣS (i.e. H_2S, HS^-, S^{2-}), low pH and dissolved oxygen, accordingly to the proximity of fluid emissions (Sarradin et al., 1999). It is the same for estimated high values for CH_4 and H_2S at the “mussels + mats” group site allowing us to hypothesize the proximity of fluid emissions. Furthermore, the estimated values of H_2S obtained in the collection area are within the range of 0-62 µM for the H_2S concentration reported for mussel beds in Eiffel Tower (Sarradin et al., 1999). The higher values recorded for temperature, CH_4 and sulfur compounds at the “mussel + mats” group site, besides contributing to free-living filament bacteria proliferation, do not seem to have any observed effect on *B. azoricus* physiological condition. Likely, *B. azoricus* as a mixotrophic organism which obtains energy not only from a dual endosymbiosis but also from suspension-feeding and can potentially regulate the relative contribution of both nutritional pathways according to external conditions (Martins et al., 2008).

4.2. Toxicological status

The interactions between the superheated fluids and cold ocean water create a dynamic system that gives, to vent organisms, periodic access to high levels of metals (Geret et al., 1998). Other studies put forward the high concentrations of metals in Lucky Strike hydrothermal vent fluids, like Cu (2 to 30 µM), Fe (70 to 920 µM) and Zn (2 to 40 µM) (Charlou et al., 2000; Douville et al., 2002) and the bioaccumulation of those metals in vent organisms, such as mussels (Colaço at al., 2006; Cosson, 2008). The pattern

Fe>Zn>Cu found for individuals from the "mussels" group is in agreement with earlier studies of *B. azoricus* metal bioaccumulation (Cravo et al., 2007; Kádár et al., 2007) and with Lucky Strike fluids composition (Douville et al., 2002). The "mussels + mats" group showed a different pattern (Zn>Fe>Cu), however it was not found any statistical difference between Zn and Fe levels in this group. Moreover, for both groups, the highest accumulation of these metals was found in the digestive gland. It has been shown that digestive gland of bivalves is a target organ for the bioaccumulation of metals (Domouhtsidou and Dimitriadis, 2000). The ability of *Bathymodiolus* sp. to capture and ingest mineral particles, including Fe, Zn and Cu sulfides (Le Pennec et al., 1985) is one pathway for bioaccumulation of metals in the digestive gland (Rousse et al., 1998). Moreover, this organ has an important role in metabolism of metals and is considered as a long-term storage tissue and thus a good indicator of persisting exposure (Hamza-Chaffai et al., 2000). The high levels of Fe found in digestive gland of "mussels" group may be a reflection of a more direct exposure of individuals to the Fe sulfide particles floating in their surrounding environment. Nevertheless, one factor that can affect metal bioaccumulation is body size (Pan and Wang, 2008). Studies developed by Boyden, (1974) regarding mussel allometry in ecotoxicology, show that highest values of trace elements are often recorded in the smallest individuals. That could be the explanation for the high Fe levels found for the "mussels" group formed by individuals presenting a smaller size than those from the "mussels + mats" group.

As metals are rather abundant at Lucky Strike hydrothermal field (Rousse et al., 1998; Douville et al., 2002; Kádár et al., 2005), their intracellular distribution between soluble and insoluble forms can be used to evaluate the toxicological significance of each metal.

However we could not show any difference for the distribution of metals between the soluble and insoluble fractions of mussels collected at the sampled locations. Metallothioneins are known to be the major metal detoxification process in mussels (Langston et al., 1998). The amounts of MT found in the tissues of *B. azoricus* from both groups were in relation with the accumulation of the analyzed metals, accordingly the higher amounts of MT were found in the digestive gland. However no significant correlation between MT and soluble metal amounts was established. Although the synthesis of MT can be enhanced by the presence of metals in the organisms there are other factors such as oxidative stress and environmental changes that can also stimulate the neosynthesis of these metalloproteins (Viarengo and Nott, 1993; Bebianno et al., 2005; Company et al., 2006). Further studies are needed regarding the relative abundance of sulfur-oxidizing filamentous bacteria at the microbial mats and the putative importance of suspension-feeding nutritional pathway in dual symbionts-bearing mussel *B. azoricus*.

5. Conclusions

The results presented in this study do not show significant physiological and toxicological evidence that emphasize the influence of associated sulfur-oxidizing filamentous bacteria on *B. azoricus* mussel. The physiological markers show a similar physiological condition between the two groups of mussels. As well, the variations found in the abiotic factors measured seem not to influence the physiological status of the two mussel groups. The presence of sulfur-oxidizing filamentous bacteria regarded as an indicator of elevated sulfide concentrations, point out a lesser diluted hydrothermal fluid at the considered area and accordingly higher levels of metals available to the organisms. However, it seems that metals are equally bioavailable for mussel in the presence and absence of sulfur-oxidizing filamentous bacteria. This work shows that, *B. azoricus* mussel seems to be well adapted to the assorted physico-chemical characteristics from the surrounding environment since it is able to manage the constant fluctuation of physico-chemical compounds.

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Table 1

Levels of Cu, Fe and Zn found in certified reference material, mussel tissue CE278 (ERM-Belgium) and lobster hepatopancreas TORT-2 (NRCC-Canada). Results as mean ± SD, in mg g^{-1} dry weight.

Certified reference material		Cu (mg g^{-1}) n= 6	Fe (mg g^{-1}) n= 8	Zn (mg g^{-1}) n= 11
CE278	Certified	-	-	0.08 ± 0.002
	Observed	-	-	0.10 ± 0.001
TORT-2	Certified	0.11 ± 0.01	0.11 ± 0.01	-
	Observed	0.11 ± 0.002	0.11 ± 0.01	-

Table 2

Lipids, carbohydrates and total proteins in whole soft tissues for the two groups studied. Results are presented as mean ± SD, in mg g^{-1} dry weight. n represents the number of analyzed mussels.

Biochemical	n	mussels	n	mussels + mats
Lipids[a]	1	138.5	1	174.8
Carbohydrates	5	29.1 ± 4.7	5	36.2 ± 9.9
Total proteins	5	889.9 ± 67.4	4	863.3 ± 60.9

[a] pooled sample

Table 3

In situ temperature measurements (ºC) and estimated values of CH_4 and H_2S (µmol l^{-1}) obtained within the sampling sites. For temperatures measurements: n= 23389, sampling period 30 sec, results are presented as mean ± SD.

	mussels			mussels + mats		
	Temperature (ºC)	CH4 estimated (µM)	$H2S_T$ estimated (µM)	Temperature (ºC)	CH4 estimated (µM)	$H2S_T$ estimated (µM)
mean ± SD	4.91 ± 0.10			5.89 ± 0.52		
minimum	4.64	0.06	<dl*	4.76	0.15	1
maximum	5.25	0.5	5	7.39	2.0	23
range	0.61			2.63		

* dl= detection limit

Table 4

Metallothionein (MT) and metal (Cu, Fe and Zn) levels in the tissues of the two groups studied. The "Mean per group" represents the mean levels of MT, Cu, Fe and Zn for each group. Results are presented as mean ± SD, in mg g^{-1} dry weight

	mussels				mussels + mats			
	MT	Cu	Fe	Zn	MT	Cu	Fe	Zn
Gill	16.7 ± 5.0	0.14 ± 0.04	0.28 ± 0.09	0.23 ± 0.06	16.9 ± 2.7	0.20 ± 0.02	0.20 ± 0.04	0.21 ± 0.04
Mantle	22.1 ± 7.7	0.08 ± 0.04	0.30 ± 0.08	0.25 ± 0.06	21.0 ± 10.7	0.07 ± 0.02	0.20 ± 0.07	0.38 ± 0.11
Foot	20.1 ± 29.7	0.10 ± 0.04	0.28 ± 0.05	0.50 ± 0.21	21.6 ± 9.2	0.05 ± 0.03	0.20 ± 0.09	0.30 ± 0.13
Digestive gland	96.8 ± 27.8	0.18 ± 0.05	0.97 ± 0.25	0.67 ± 0.14	114.2 ± 38.2	0.20 ± 0.09	0.65 ± 0.18	0.65 ± 0.26
Remaining	18.0 ± 1.4	0.07 ± 0.01	0.48 ± 0.19	0.34 ± 0.11	15.9 ± 2.9	0.07 ± 0.04	0.20 ± 0.04	0.19 ± 0.08
Mean per group	21.5 ± 0.2	0.13 ± 0.03	0.32 ± 0.08	0.28 ± 0.06	19.1 ± 0.2	0.11 ± 0.04	0.19 ± 0.05	0.21 ± 0.06