



**HAL**  
open science

## Effects of Temperature on Scope for Growth and Accumulation of Cd, Co, Cu and Pb by the Marine Bivalve

Valentine K. Mubiana, Ronny Blust

► **To cite this version:**

Valentine K. Mubiana, Ronny Blust. Effects of Temperature on Scope for Growth and Accumulation of Cd, Co, Cu and Pb by the Marine Bivalve. *Marine Environmental Research*, 2007, 63 (3), pp.219. 10.1016/j.marenvres.2006.08.005 . hal-00501888

**HAL Id: hal-00501888**

**<https://hal.science/hal-00501888>**

Submitted on 13 Jul 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Accepted Manuscript

Effects of Temperature on Scope for Growth and Accumulation of Cd, Co, Cu and Pb by the Marine Bivalve *Mytilus edulis*

Valentine K. Mubiana, Ronny Blust

PII: S0141-1136(06)00178-4  
DOI: [10.1016/j.marenvres.2006.08.005](https://doi.org/10.1016/j.marenvres.2006.08.005)  
Reference: MERE 3067

To appear in: *Marine Environmental Research*

Received Date: 4 January 2006  
Revised Date: 9 August 2006  
Accepted Date: 31 August 2006



Please cite this article as: Mubiana, V.K., Blust, R., Effects of Temperature on Scope for Growth and Accumulation of Cd, Co, Cu and Pb by the Marine Bivalve *Mytilus edulis*, *Marine Environmental Research* (2006), doi: [10.1016/j.marenvres.2006.08.005](https://doi.org/10.1016/j.marenvres.2006.08.005)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 Effects of Temperature on Scope for Growth and Accumulation of Cd, Co, Cu and

2 Pb by the Marine Bivalve *Mytilus edulis*

3

4

5

6

7

8

9

10

11 Valentine K. Mubiana and Ronny Blust

12

13

14

15

16

17

ACCEPTED MANUSCRIPT

## 1 Abstract

2 In many aquatic organisms including *Mytilus edulis*, the role of temperature on  
3 bioaccumulation of metals is still not clearly understood. In this study, uptake and  
4 accumulation of Cu, Co, Cd and Pb in mussels were investigated at different  
5 temperatures (6 - 26 °C). Results from exposure of isolated gills showed a positive  
6 relationship between temperature and metal uptake. But in whole organism experiments,  
7 only the accumulations of non- essential metals (Cd, Pb) showed a similar trend while  
8 the two essential metals Co and Cu were either independent or inversely related to  
9 temperature, respectively. With exception of Cu, elimination process appeared to be  
10 independent of temperature. The study also showed that neither changes in scope for  
11 growth (SFG) of mussels nor chemical speciation could fully account for the observed  
12 temperature-effects. Overall, these results suggest that fundamentally (i.e. at epithelial  
13 membranes), temperature-effects on uptake are largely due to changes in solution  
14 chemistry and physical kinetics, which favours higher uptake at high temperature. But at  
15 whole organism level, complex physiological responses appears to mask the  
16 relationship, particularly for biologically essential metals like copper.

17 **Key words;** Mussels, Heavy metals, Bioaccumulation, Temperature, Speciation, Scope  
18 for Growth.

19

## 20 1. Introduction

21 Bioaccumulation of heavy metals in aquatic organisms such as mussels depends not  
22 only on environmental concentrations but also on a variety of biological and  
23 environmental factors. The common marine mussel *M. edulis* is widely used in  
24 monitoring metal pollution in coastal and estuarine waters (e.g. Goldberg, Koide,  
25 Hodge, Flegal, & Martin, 1983; De Kock & Kramer, 1994). Therefore, understanding

1 the possible roles of temperature and other environmental factors on metal uptake and  
2 accumulation in these biomonitors is critical in order to correctly relate tissue  
3 concentrations to those in the surrounding environment. Temperature affects both metal  
4 chemistry in seawater (Byrne, Kump, & Cantrell, 1988) and physiology of mussels  
5 (Dame, 1996). Temperature affects metal chemistry by changing chemical speciation,  
6 pH, solubility, reaction rates or physical kinetics (Byrne et al., 1988; Blust, Van  
7 Ginneken, & Declair, 1994). Theoretical calculations of chemical speciation in seawater  
8 indicate that changes in temperature and pH have most effects on strongly hydrolysed  
9 and carbonate complexes and less effect on chlorides and free metal ions (Byrne et al.,  
10 1988). Though it is widely known that uptake is largely controlled by the free metal ion,  
11 some studies have also shown significant contribution of other species, particularly  
12 metals bound to weak complexes (i.e. Campbell, 1995; Hudson, 1998; Lorenzo, Beiras,  
13 Mubiana, & Blust, 2005). Chemical speciation indicates that increase in temperature  
14 generally results in increase in the concentrations and activities of bioavailable metal  
15 forms, therefore, enhance uptake, at least in theory.

16 Like most aquatic organisms, mussels cannot regulate their body temperature in  
17 accordance with surrounding environment, as a result most physiological and  
18 biochemical processes are temperature dependent (Dame, 1996). One measure of  
19 animal's integrated physiological status is Scope for growth (SFG), which is essentially  
20 an energy balance equation of an individual determined from energy absorbed from  
21 ingested food items minus the energy lost through respiration and excretion (Widdows,  
22 1978; Widdows & Johnson, 1988). During the past 15 years, many studies have applied  
23 SFG approach as a quantitative measure of the impact of environmental stress due to i.e.  
24 salinity and temperature (Widdows, 1978; Sobral & Widdows, 1997; Smaal &  
25 Widdows, 1994; Vercauteren & Blust, 1999) and chemical pollution (Widdows &

1 Johnson, 1988; Widdows, Nasci, & Fossato, 1997; Widdows, Donkin, Staff,  
2 Matthiessen, Law, Thain et al., 2002; Smaal & Widdows, 1994). The role of  
3 physiological energetics such as SFG in ecotoxicology is discussed in detail in a review  
4 by Widdows & Donkin (1991). Smaal & Widdows (1994) outlined the justification for  
5 use of SFG as an integrated response in biological monitoring programmes.

6 The role of temperature on metal uptake and accumulation in marine mussels has  
7 not been well studied. This is despite the fact that mussels are important biomonitoring  
8 organisms. Furthermore, there seem to be no general consensus among the few available  
9 studies on the subject. Carpene & George (1981) found no temperature effects on Cd  
10 uptake in isolated gills. However, lack of prior temperature acclimation of mussels used  
11 in that study may have influenced the results. In whole organism experiments, Phillips  
12 (1976) detected no temperature effects on accumulation of Cd, Cu, Pb and Zn except at  
13 low salinity (<18 ppt). Fischer (1986) also observed that Cd accumulation by mussels  
14 was only significantly decreased at very low temperatures (<7 °C). In both studies  
15 temperature exposures were coupled with different salinity treatments, therefore it is not  
16 clear whether salinity or interaction between the two may have influenced the results.  
17 Some recent studies have reported uptake that is dependent and increases with increase  
18 in temperature (Wang, Chuang, & Wang, 2005). Baines, Fisher, & Kinney (2005) also  
19 found a positive relationship between assimilation efficiency of dietary metals with  
20 temperature. These studies are consistent with results involving many different  
21 organisms, which tend to show a positive correlation between temperature and metal  
22 uptake, accumulation or toxicity (McLusky, Bryant, & Campbell, 1986; Blust et al.,  
23 1994; Bervoets, Blust, & Verheyen, 1996; Chowdhury & Blust, 2001).

24 The objective of the current study was to investigate the influence of temperature on  
25 uptake and accumulation of two types of metals i.e. Co, Cu as biologically essential

1 metals and Cd and Pb as non- essential metals. Accumulation kinetics of these metals  
2 were determined in long-term exposure experiments. Short-term exposures involving  
3 isolated gills were also conducted. These experiments involved exposure of gills to  
4 either single metal solutions or metal mixtures. The two treatments were intended to  
5 check whether there were significant interactions among metals during uptake. Isolated  
6 gills experiments minimised the influence of animal physiology and hence provide a  
7 better insight into the fundamental mechanisms and processes involved. The main  
8 consequences of temperature changes namely, metal speciation, pH and dissolved  
9 oxygen were also determined. SFG was determined as an integrated measure of the  
10 animal physiological status in response to differences in ambient temperature.

11

## 12 **2. Materials and methods**

### 13 *2.1. Test animals*

14 Adult mussels of 40 – 50 mm shell length were collected from Eastern Scheldt (The  
15 Netherlands), a former estuary turned into a semi-enclosed marine bay after construction  
16 of dams across river channels in the early 1980s. This area is largely a marine tidal bay  
17 with relatively low levels of metal pollution (Mubiana, Qadah, Meys, & Blust, 2005).

### 18 *2.2. Exposure medium*

19 Artificial seawater was prepared by dissolving 35 g/L sea salt *hw Sea Salt*  
20 *professional* (Wiegandt GmbH, Germany) in deionised water. Elemental composition of  
21 this salt is well described and contains only traces of heavy metals i.e. Cu (200 ng/kg),  
22 Pb (19 ng/kg), Co (3 ng/kg) and Cd (0.1 ng/kg) (<http://www.hw-wiegandt.de>). Metal  
23 exposure solutions were then prepared by dissolving high purity salts of CdCl<sub>2</sub>, CoCl<sub>2</sub>,  
24 CuCl<sub>2</sub> and PbNO<sub>3</sub> either as a mixture of all four metals or single metal solutions.  
25 Exposure concentrations (see Table 1) were neither too low for measurable uptake nor

1 too high to be environmentally relevant. However, in view of the short exposure times  
2 for isolated gills (1-h), relatively high exposure concentrations (0.5  $\mu\text{M}$ ) were used in  
3 order to achieve measurable uptake in the gills.

4 Prepared solutions were always left for 48-hrs to equilibrate, analysed for metal  
5 concentrations and if necessary additional metals were added. In long-term exposures,  
6 water was changed regularly to avoid significant drop in concentrations. Metal  
7 concentrations in water samples were analysed with ICP-AES (Liberty series II, Varian  
8 Australia) and as shown in Table 1 metal concentrations did not fluctuate by more than  
9 10 % within or among tanks. Metal speciation in prepared seawater as a function of  
10 temperature and  $\text{pH}$  was calculated using chemical speciation model Visual MINTEQ  
11 2.32 (Gustafsson, 2005). Metal speciation calculations were only performed for the  
12 water that was used in isolated gills experiments. Concentration of DOC in the exposure  
13 water (in gills experiments) was found to be below method detection limits (0.01 mg/l)  
14 and was therefore not included in the speciation model.

### 15 2.3. *Whole organism experiments*

16 After sampling, animals were transported on ice to the laboratory and on arrival 20  
17 and 14 individuals were chosen randomly for metal analysis and SFG measurements,  
18 respectively. Remaining mussels were divided into three groups of about 250 mussels  
19 and each group was placed in 500 L tanks. Initially, temperature was set at 13  $^{\circ}\text{C}$  (field  
20 temperature) then after a week and during two weeks it was slowly adjusted to obtain 6  
21  $^{\circ}\text{C}$ , 16  $^{\circ}\text{C}$  and 26  $^{\circ}\text{C}$  after which acclimation was then continued for another week.  
22 Mussels were always fed 10 mg /individual of enriched yeast cells (Lanzy PZ, Inve,  
23 Belgium) twice a week. To minimise organic matter loading in the experimental tanks,  
24 feeding of mussels was in separate tanks during 3-hrs with conditions identical to  
25 experimental tanks. Furthermore, organic carbon was monitored every 3 days using a



1 TOC 5000 Analyzer (Shimadzu) fitted with a 0.8 mm diameter suspended particle  
2 sampler. Both DOC and POC varied insignificantly with values consistently below 0.1  
3 mg/L and 0.01 mg/L, respectively.

4 At time intervals 7 – 9 mussels were collected from each tank for metal analysis. On  
5 day 28, animals were transferred to clean seawater for elimination during 28 days. In the  
6 first 14 days water was replaced every three days and then every six days for the  
7 remaining period. After sampling, each animal was partially opened and the inside  
8 cavity sprayed with purified water to flush-out trapped seawater. They were then  
9 dissected and soft tissues were individually placed in pre-weighed polypropylene vials.  
10 The tissues were freeze-dried at  $-60\text{ }^{\circ}\text{C}$  for 72-h, after which tissue dry weight was  
11 determined.

12 Five ml high purity concentrated  $\text{HNO}_3$  and 0.25 ml of 27%  $\text{H}_2\text{O}_2$  were then added  
13 and the digest was further destructed by microwave heating according to Blust, Vander  
14 Linden, Verheyen, & Declair (1988). Samples were heated in four steps of five minutes  
15 at 80, 160, 240 and 320 watts, and then diluted with purified water to bring acid  
16 concentration to about 2%. Metal concentrations in the final solutions were measured  
17 with ICP-MS (Ultra Mass 700, Varian Australia) employing yttrium and indium as  
18 internal standards. As quality control, three reference samples (mussel tissue CRM  
19 278R, IRMM, Geel Belgium) and three preparation blanks were included in each 40-  
20 sample rack and were carried along in every step of the analysis. Recoveries for all four  
21 metals in the reference samples were consistently within 96 – 101 % of the certified  
22 values.

#### 23 *2.4. Measurements of scope for growth*

24 Scope for growth measurements were conducted according to a procedure adopted  
25 from Widdows & Johnson (1988) and Widdows & Staff (1997). Unless stated, all the

1 constants used in the equations were adopted from these references. The first SFG  
 2 parameter determined was clearance rate (CR). Mussels were placed in clean filtered  
 3 seawater for 1-hr to flush-out any particles enclosed inside shell valves and then  
 4 individually placed in 2-L seawater, which was previously filtered (0.45  $\mu\text{m}$ ) and then  
 5 spiked with yeast cells (Lanzy PZ, Inve, Belgium) at 50,000 cells/ml. The vessels were  
 6 aerated to provide homogenous distribution of cells and then every 15 minutes for 90  
 7 minutes, 10 ml water samples were taken for cell counting with a Coulter Counter. By  
 8 definition, CR is the volume of water cleared of particles per gram mussel per hour and  
 9 was calculated according to the expression:

$$10 \quad \text{CR (L g}^{-1} \text{ h}^{-1}) = \text{volume of water (L) x } (\log_e C_1 - \log_e C_2) / \text{DW x time interval (h)}$$

11 *Where*  $C_1$  and  $C_2$  are cell concentrations at the beginning and end of each time  
 12 increment and  $DW$  is soft tissue dry weight (g). CR during each time interval was  
 13 calculated and for each individual the highest CR or ‘maximum clearance rate’ (MCR)  
 14 was converted into energy equivalent according to the formula:

$$15 \quad \text{Energy absorbed (J/h/g)} = \text{MCR (L g}^{-1} \text{ h}^{-1}) \times \text{POM} \times 23 \text{ J mg}^{-1} \times 0.5$$

16 *Where*, POM is concentration of yeast cells (mg/L) with energy of 23 J  $\text{mg}^{-1}$  ash free  
 17 dry weight (obtained from the manufacturer, Inve, Belgium) and 0.5 is absorption  
 18 efficiency from yeast cells, which was calculated from ash free dry weight of ingested  
 19 cells and faeces produced by the mussels.

20 After measuring CR, each mussel was then transferred into a 500-ml respirometer  
 21 filled with clean filtered (0.45  $\mu\text{m}$ ) water and fitted with an oxygen electrode connected  
 22 to a computer, which recorded dissolved oxygen over time (90 minutes). Measured  
 23 respiration rates were then converted into energy equivalent according to the formula:

$$24 \quad \text{Energy respired (J/h/g)} = \text{Respiration rate } (\mu\text{moles O}_2 \text{ g}^{-1} \text{ h}^{-1}) \times 0.456$$

1       Where  $0.456 \text{ J } \mu\text{mole}^{-1} \text{ O}_2$  is the heat equivalent of oxygen uptake.

2       Energy excreted was calculated from ammonia produced during respiration  
3       measurements. Water samples were taken before and after incubation and were filtered  
4       ( $0.45 \mu\text{m}$ ) and then analysed for ammonia concentration according to the phenol-  
5       hypochlorite method of Solorzano (1969). Ammonia produced by each mussel was then  
6       converted into energy equivalents as follows:

7       Energy excreted (J/h/g) = Ammonia excretion rate ( $\mu\text{moles NH}_3\text{-N g}^{-1} \text{ h}^{-1}$ ) x 0.349

8  
9       Where excretion of  $1 \mu\text{mole NH}_3\text{-N h}^{-1}$  is equivalent to energy loss of  $0.349 \text{ J h}^{-1}$ .

10      Finally SFG was calculated as:

11                       $\text{SFG} = \text{Energy absorbed} - (\text{Energy respired} + \text{Energy excreted})$

## 12      2.5. *Metal uptake in isolated gills*

13       For each temperature treatment, three groups of gills consisting of 14 – 15 replicates  
14       were obtained from fully temperature acclimated mussels. After extraction, gills from  
15       one group were rinsed in clean seawater and individually incubated for 1-h in 300 ml of  
16       clean seawater (controls). The second and third groups were incubated under the same  
17       conditions and at  $0.5 \mu\text{M}$  single and metal mixture solutions, respectively.

18       After incubation gills were washed three times in 300 ml clean seawater and then in  
19       purified water. This procedure was found to be sufficient to remove loosely adsorbed  
20       metals. It is generally accepted that strongly adsorbed metals are associated more with  
21       internalised than external metal fraction (Endo, Kimura, & Sakata, 1998). After washing  
22       gills were placed in pre-weighed polypropylene tubes and freeze –dried at  $-60 \text{ }^\circ\text{C}$  during  
23       72-hrs. Dry samples were then digested in 1 ml  $\text{HNO}_3$  and 0.05 ml  $\text{H}_2\text{O}_2$  as described  
24       earlier and reference material and preparation blanks were also included. Metal  
25       concentrations in diluted solutions were then analysed with ICP-MS as earlier described.

## 1 2.6. Modelling bioaccumulation

2 Metal accumulation in mussels was best fitted by linear regressions while  
3 elimination data was best fitted by single exponential elimination model (Newman,  
4 1995):

$$5 \quad C_{\text{mussel}} = a + b * (1 - e^{-k_e t})$$

6 where  $C$  is metal concentration ( $\mu\text{g/g}$ ) in soft tissues,  $a$  and  $b$  are model estimates,  $k_e$   
7 is elimination rate constant ( $\text{day}^{-1}$ ) and  $t$  is time (days). The data were fitted in the  
8 software GraphPad Prism 4.0.

9

## 10 3. Results

### 11 3.1. Scope for growth

12 Figure 1a shows soft tissue dry weights (mean of 14 – 20 mussels) at the time of  
13 sampling (field) and at the end of the experiment. Since sample sizes were small data  
14 were tested for differences using Kruskal-Wallis ANOVA and results showed no  
15 significant differences among the four groups. Figure 1b & c shows MCR and  
16 respiration rates of mussels after sampling (field) and after acclimation (crossed bars)  
17 and at end of experiment (grey bars). Both data were subjected to Kruskal-Wallis test  
18 with Dunn's post-hoc and results showed higher MCR under field conditions and there  
19 were significant differences among temperature treatments (as shown in the Figures).  
20 Respiration rate was only significantly lower at 6 °C. Both respiration rate and MCR  
21 showed no differences between measurements performed after acclimation and after  
22 metal exposures (results not shown for clarity), similar trend was shown for energy  
23 excreted (as ammonia) but there were differences among temperature treatments with  
24 highest energy excreted at 26 °C ( $1.26 \pm 0.2 \text{ J h}^{-1} \text{ g}^{-1}$ ) followed by 16 °C ( $0.23 \pm 0.05 \text{ J h}^{-1}$ )

1  $\text{g}^{-1}$ ), then field ( $0.13 \pm 0.03 \text{ J h}^{-1} \text{ g}^{-1}$ ) and the least was at  $6 \text{ }^\circ\text{C}$  ( $0.05 \pm 0.01 \text{ J h}^{-1} \text{ g}^{-1}$ ).  
2 Energy excreted contributed less 1 % towards SFG while clearance rate (or food  
3 acquisition) contributed most, consistent with literature (Sobral & Widdows, 1997;  
4 Widdows & Staff, 1997). Finally, as shown in Figure 1d, SFG was highest under field  
5 conditions, lowest at  $26 \text{ }^\circ\text{C}$  and there was no difference between  $6 \text{ }^\circ\text{C}$  and  $16 \text{ }^\circ\text{C}$ .

### 6 *3.2 Metal accumulation in whole organisms*

7 Figure 2 shows metal concentrations in mussels at sampling (day 0), during metal  
8 exposure (day 28 – 56) and during depuration (day 56 – 84). In order to determine  
9 whether acclimation and temperature had effect on metal concentrations, day 0 and day  
10 28 data in all three temperature treatments were subjected to ANOVA. Results showed  
11 that overall there were significant differences ( $p < 0.05$ ) as shown in the figure and post-  
12 hoc test results for specific differences are summarized in Table 2. Metal accumulation  
13 increased linearly with time and in order to evaluate the effect of temperature, slopes of  
14 the linear lines were compared using *F-statistics*. Results showed significant differences  
15 except for Co concentration whose pooled slope was calculated as 0.046. Post-hoc test  
16 (Table 2) showed that slopes increased with increase in temperature for the non-  
17 essential metals Cd and Pb while the slope for Cu decreased with temperature.

18 Metal elimination data were best fitted using single exponential elimination kinetics  
19 (Newman, 1995) and results showed no effect of temperature (*F-statistics*,  $p > 0.05$ ) on  
20 the elimination rate constants ( $k_e$ ) with exception of Cu, which showed lower  $k_e$  at  $6 \text{ }^\circ\text{C}$   
21 (Table 2). Finally net metal accumulation calculated as a difference between pre-  
22 exposure (day 28) and after depuration (day 84) showed that the two essential metals Co  
23 and Cu did not register significant net accumulation (see Figure 2).

### 24 *3.3. Metal uptake in isolated gills*

1 Figure 3 shows metal concentrations in control gills and those exposed to either  
2 single or metal mixtures at three test temperatures. The data were analysed for  
3 significant differences among the groups using Kruskal-Wallis ANOVA with post-hoc  
4 test. For all four metals, uptakes in gills exposed to metals were higher than the controls,  
5 except for Cu at 6 °C. For the purpose of clarity only post-hoc test results comparing  
6 uptake from single metal and metal mixtures are shown (*p-values* on top of bar graphs).  
7 As shown in Figure 3, uptake from single metal solutions were consistently higher than  
8 from metal mixtures, except in a few cases i.e. Co at 6 °C and 16 °C, Cu at 16 °C and 26  
9 °C and Pb at 6 °C.

10 Inserted graphs in Figure 3 show metal uptakes in gills exposed to single metal  
11 solutions. The values were calculated as averages ( $\pm$ SD) of the differences between  
12 mean controls and concentration in individual gills. The data were subjected to Kruskal-  
13 Wallis ANOVA and results as shown in Table 3 indicated significant differences,  
14 confirming that uptake was higher at higher temperature.

#### 16 **4. Discussion**

17 The study showed that with exception of Cu, tissue concentrations decreased during  
18 acclimation as was expected due to low background levels of metals in the acclimation  
19 water. However, the decreases appeared to depend on temperature of acclimation, with  
20 animals in the lowest temperature losing most metals. This effect was in contrast with  
21 later results during elimination phase where no effects of temperature were observed  
22 with the exception of copper (Figure 2 & Table 2). Therefore, temperature-effects during  
23 acclimation appeared to be an initial response, which disappeared once animals were  
24 fully acclimated and had been exposed to higher metal concentrations. It is not clear  
25 what kinds of processes are responsible for such responses. However, it is reasonable to

1 assume that some physiological and biochemical changes related to temperature  
2 acclimation may be somehow responsible for influencing metal loss during the early  
3 stages of acclimation. These types of effects may be relevant for inter-tidal organisms  
4 such as mussels, which are often exposed to temperature fluctuations during tidal cycle.

5 The general lack of temperature effects on elimination especially for non- essential  
6 metals Cd and Pb is consistent with some studies on marine bivalves (Amiard, Amiard-  
7 Triquet, Berthet, & Métayer, 1986, 1987; Hutchins, Stupakoff, Hook, Luoma, & Fisher,  
8 1998), echinoderms (Hutchins, Teyssié, Boisson, Fowler, & Fisher, 1996) and Japanese  
9 eel (Yang & Chen, 1996). In field studies seasonal variations in tissue metal  
10 concentrations may to some extent imply temperature effects. However, many studies  
11 seem to indicate that most of the seasonal variability may well be due to changes in  
12 animal body weight, since total body burdens remained largely unchanged (Cossa,  
13 Bourget, & Piuze, 1979; Amiard et al., 1986; Mubiana et al., 2005). However, size  
14 related factors (i.e. growth rate, age and body size), can still significantly influence not  
15 only concentrations but can also affect bioaccumulation of metals in marine mussels  
16 (Mubiana, Vercauteren, & Blust (*in press*). Therefore, changes in body weight during  
17 long-term exposure experiments can have an influence on accumulated metals.  
18 However, in this particular study, differences in tissues weights among the treatments  
19 before and after exposures were not significantly different (Fig 1a). Therefore, change in  
20 tissue weight could not have been a significant factor responsible for the observed metal  
21 accumulation patterns, other factors must be evoked.

22 As demonstrated in this study and evidence from literature, it seems more likely that  
23 in the long-term, temperature has little or no influence on elimination of most metals,  
24 except perhaps for some essential metals such as Cu. In the case of Co, the lack of  
25 temperature-effects on both accumulation and elimination in whole organisms despite

1 clear temperature effects at isolated gills level is further indication of physiological  
2 regulation. It is well known that most organisms are capable of controlling internal  
3 concentrations of essential metals up to a certain threshold level (Rainbow, 1997, 2002).  
4 Animals regulate metal concentrations either in response to external metals exposures  
5 and /or in response to environmental factors including temperature or due to attainment  
6 of critical body levels (Rainbow, 2002).

7 Lack of clear evidence for the effect of temperature on the elimination of metals is  
8 surprising since temperature has such a profound effect on physiology and metabolism  
9 of marine mussels (Hawkins & Bayne, 1992; Dame, 1996). Furthermore, in almost all  
10 aquatic invertebrates many essential metals are known to play a variety of important  
11 roles in physiological and metabolic processes (Rainbow, 1997, 2002). Rainbow (2002)  
12 described different types of metal accumulation patterns in invertebrates. It is clear that  
13 the fate of metabolically available metals in an organism can involve direct excretion or  
14 storage in a detoxified form prior to excretion. The role of changes in physiology in all  
15 these processes leading to metal excretion is still not well understood in many  
16 organisms. Therefore, the general role (or lack of involvement) of physiology and  
17 metabolism on metal elimination process deserves further investigation. Unlike  
18 elimination, there is a bulk of literature showing evidence for temperature effect on  
19 metal uptake, accumulation and toxicity (i.e. McLusky et al., 1986; Blust et al., 1994;  
20 Hutchins et al., 1996, 1998; Yang & Chen, 1996).

21 In seawater, temperature influences a variety of physiochemical factors and the  
22 important ones include changes in pH and chemical speciation of metals (Byrne et al.,  
23 1988). As shown in Figure 4 and consistent with literature, temperature showed little  
24 effect on metal speciation, particularly on the free metal ion activity, which is the most  
25 bioavailable form for most metals and organisms (Campbell, 1995; Hudson, 1998).



1 Generally, increase in temperature results in the increase in free metal ion activity and a  
2 few other metal species like carbonates. Some studies have indicated that besides free  
3 metal ions, other metal forms such as  $\text{OH}^-$  and  $\text{CO}_3$  complexes can somehow be  
4 bioavailable to organisms (Hudson, 1998; Laporte, Truchot, Ribeyre, & Boudou, 1997).  
5 Therefore, although temperature-effects on metal speciation were generally modest at  
6 least within environmentally relevant temperature ranges, nevertheless, it was expected  
7 that increase in temperature would generally favour higher uptake. Besides speciation  
8 effects, changes of most physiochemical factors such as chemical reaction and diffusion  
9 rates would favour higher uptake with increase in temperature (McLusky et al., 1986;  
10 Blust et al., 1994; Bervoets, Blust, & Verheyen, 1996; Chowdhury & Blust, 2001). In  
11 the case of pH, since it is usually positively correlated with temperature (Byrne et al.,  
12 1988), increase in temperature was expected to decrease metal uptake. However, since  
13 seawater has high buffering capacity, only moderate changes in pH between 6 – 26 °C  
14 were observed (Table 1). On this basis pH was not expected to be a major factor  
15 determining metal uptake or accumulation in this study.

16 The range of SFG determined in this study is comparable with literature concerning  
17 *M. edulis*. As shown in Figure 1, clearance rate of about  $7 \text{ L g}^{-1} \text{ h}^{-1}$  corresponding to  
18 SFG of nearly  $14 \text{ J g}^{-1} \text{ h}^{-1}$  measured under field conditions, which are within a typical  
19 range for mussels from clean environment (i.e. Widdows & Staff, 1997; Smaal &  
20 Widdows, 1994). SFG measurements representing field conditions (day 0) are consistent  
21 with the fact that Eastern Scheldt is well known for its favourable conditions for  
22 shellfish farming in The Netherlands. However, after laboratory acclimation SFG  
23 decreased almost by half at 6 °C and 16 °C. This decrease was mainly due to low  
24 clearance rate, which is not surprising considering the obvious differences between field  
25 and laboratory conditions in terms of food quality and quantity. Negative SFG at 26 °C

1 implied that animals were utilizing energy reserves, mainly because high temperature  
2 seriously inhibited clearance rate. As demonstrated in other studies (i.e. Sobral &  
3 Widdows, 1997; Smaal & Widdows, 1994) clearance rate (or food acquisition) is the  
4 most sensitive parameter for SFG. The study showed that apart from the initial drop in  
5 SFG during acclimation, temperature only influenced SFG at the highest temperature  
6 while there was no significant difference between 6 – 16 °C. This is consistent with  
7 expectations, temperate *Mytilus spp.* are well known for their ability to tolerate very low  
8 temperatures (<10 °C) whereas very high temperatures (generally >22 °C) can have very  
9 serious detrimental effects on mussels (Seed & Suchanek, 1992; Dame, 1996). In this  
10 respect, the study has shown that in terms of energy budgets, mussels are  
11 physiologically more tolerant to very low temperatures. The study has also shown that  
12 SFG can be a useful tool to determine stress from high temperature exposures such as  
13 occasionally experienced in the summer periods.

14 SFG is essentially an integration of many complex physiological processes some of  
15 which can be directly or indirectly linked to metal uptake or accumulation in mussels.  
16 For example, change in clearance rate has the potential to affect exposure to dissolved  
17 metals, but the nature of the relationship is not yet clear. Theoretically, increased  
18 clearance rate implies increased contact with waterborne metals and thus enhanced  
19 uptake and accumulation. However, Blackmore and Wang (2003) observed low uptake  
20 among mussels with high filtration rates, though those results may have been influenced  
21 by other factors. Respiration rate also has the potential to affect metal content of  
22 mussels, Wang et al. (2005) found uptake of Cd and Zn to decrease with decrease in  
23 respiration rate. In this study, none of the SFG parameters could be directly correlated  
24 with observed metal uptake or accumulation among the temperature treatments. This  
25 does not in anyway exclude effects of SFG on metal accumulation, what the results

1 highlight is the fact that temperature related physiological responses are complex and  
2 many of them can directly or indirectly influence metal uptake and accumulation.  
3 Further research is required especially including wide differences in terms of SFG, in  
4 order to better assess the impact or lack of SFG on metal accumulation in mussels.

5 The study has shown that when considered at the solution-organism interface such  
6 as isolated gills, temperature effects on both essential and non-essential metals is largely  
7 controlled by solution chemistry and physical kinetics, which tend to favour higher  
8 uptake with increase in temperature (Byrne et al., 1988; Blust et al., 1994; Simkiss,  
9 1995). However, at whole organism level, complex physiological responses tend to  
10 mask the relationship, particularly for biologically essential metals like copper, which  
11 are subject to regulation over a certain range and conditions (Rainbow, 2002; Amiard et  
12 al., 1986, 1987).

#### 14 **Acknowledgements**

15 We are very thankful to the Belgian Federal Office for Science, Technical and Cultural  
16 Affairs (OSTC) who partially financed this work through the CALMARS project  
17 contract DWTC-EV/05/04C. We are also thankful to our former post-graduate student  
18 Mr. Richard Juma for his contribution in the preliminary experiments.

#### 20 **References**

- 21 Amiard, J. C., Amiard-Triquet, C., Berthet, B., & Métayer, C. (1986). Contribution to  
22 the ecotoxicological study of cadmium, lead, copper and zinc in the mussel *Mytilus*  
23 *edulis*: I. Field study. *Marine Biology*, 90, 425-431.
- 24 Amiard, J. C., Amiard-Triquet, C., Berthet, B. & Metayer, C. (1987). Comparative study  
25 of the patterns of bioaccumulation of essential (Cu, Zn) and non-essential (Cd, Pb)

- 1 trace metals in various estuarine and coastal organisms. *Journal of Experimental*  
2 *Marine Biology and Ecology*, 106, 73-89.
- 3 Baines, S. B, Fisher, N. S., & Kinney, E. L. (2005). Influence of temperature on dietary  
4 metal uptake in Arctic and temperate mussels. *Marine Ecology Progress Series*, 289,  
5 201-213.
- 6 Bervoets, L., Blust, R., & Verheyen, R. (1996). Effects of temperature on Cadmium and  
7 Zinc uptake by the Midge Larvae *Chironomus riparius*. *Archives of Environmental*  
8 *Contamination and Toxicology*, 31, 502-511.
- 9 Blackmore, G. & Wang, W-X. (2003). Inter-population differences in Cd, Cr, Se, and Zn  
10 accumulation by the green mussel *Perna viridis* acclimated at different salinities.  
11 *Aquatic Toxicology*, 62, 205-218.
- 12 Blust, R., Ginneken, L. V., & Decler, W. (1994). Effect of temperature on the uptake of  
13 copper by the brine shrimp, *Artemia franciscana*. *Aquatic Toxicology*, 30, 343-356.
- 14 Blust, R., Vander Linden, A., Verheyen, E., & Decler W. (1988). Evaluation of  
15 microwave-heating digestion and graphite-furnace atomic-absorption spectrometry  
16 with continuum source background correction for the determination of Fe, Cu and  
17 Cd in brine shrimp. *Journal of Analytical Atomic Spectrometry*, 3, 387-393.
- 18 Byrne, R. H., Kump, L. R., Cantrell, K. J. (1988). The influence of temperature and pH  
19 on trace metal speciation in seawater. *Marine Chemistry*, 25, 168-181.
- 20 Campbell, P. G. C. (1995). Interactions between Trace Metals and Aquatic Organisms:  
21 A Critique of the Free-ion Activity Model. In A. Tessier & D.R. Turner (Eds.), *Metal*  
22 *Speciation and Bioavailability in Aquatic Systems* (pp. 45-97). Chichester: John  
23 Wiley & Sons Ltd.
- 24 Carpena, E. & George, S. G. (1981). Absorption of cadmium by gills of *Mytilus edulis*  
25 (*L.*). *Molecular Physiology*, 1, 23-34.
- 26 Chowdhury, M. J. & Blust, R. (2001). Effect of temperature on the uptake of waterborne  
27 strontium in the common carp, *Cyprinus carpio (L.)*. *Aquatic Toxicology*, 54, 151-  
28 160.
- 29 Cossa, D., Bourget, E., & Piuze, J. (1979). Sexual maturation as a source of variation in  
30 the relationship between cadmium concentration and body weight of *Mytilus edulis*  
31 *L.* *Marine Pollution Bulletin*, 10, 174-176.

- 1 Dame, R.F. (1996). *Ecology of marine bivalves an ecosystem approach* (pp. 19-26).  
2 Boca Raton: CRC Press.
- 3 De Kock, W. C., & Kramer, K. J. M. (1994). Active Biomonitoring (ABM) by  
4 Translocation of Bivalve Molluscs, In K.J.M. Kramer (Ed.), *Biomonitoring of*  
5 *Coastal waters and Estuaries* (pp. 51-84). Boca Raton: CRC Press.
- 6 Endo, T., Kimura, O., & Sakata, M. (1998). Cadmium uptake from apical membrane of  
7 LLC-PK1 cells via inorganic anion exchanger. *Pharmacology and Toxicology*, 82,  
8 230-235.
- 9 Fischer, H. (1986). Influence of temperature, salinity, and oxygen on the cadmium  
10 balance of mussels *Mytilus edulis*. *Marine Ecology Progress Series*, 32, 265-278.
- 11 Goldberg, E. D., Koide, M., Hodge, V., Flegal, A. R., & Martin, J. H. (1983). US  
12 Mussel Watch: 1977–1978 results on trace metals and radionuclides. *Estuarine*  
13 *Coastal and Shelf Science*, 16, 69-93.
- 14 Gustafsson, J. P. (2005). *Visual MINTEQ ver. 2.32* (last accessed 31/10/2005).  
15 <http://www.lwr.kth.se/English/OurSoftware/vminteq/index.htm>.
- 16 Hawkins, A. J. S. & Bayne, B. L. (1992). Physiological interrelations, and the regulation  
17 of production. In E. Gosling (Ed.), *The Mussel Mytilus: Ecology, physiology,*  
18 *genetics and culture* (pp. 171-212). Amsterdam: Elsevier.
- 19 Hudson, R. J. M. (1998). Which aqueous species control the rates of trace metal uptake  
20 by aquatic biota? Observations and predictions of non-equilibrium effects. *Science of*  
21 *The Total Environment*, 219, 95–115.
- 22 Hutchins, D. A., Stupakoff, I., Hook, S., Luoma, S. N., & Fisher, N. S. (1998). Effects of  
23 arctic temperature on distribution and retention of the nuclear waste radionuclides  
24 <sup>241</sup>AM, <sup>57</sup>Co, and <sup>137</sup>Cs in the bioindicator bivalve *Macoma balthica*. *Marine*  
25 *Environmental Research*, 45, 17-28.
- 26 Hutchins, D. A., Teysié, J.-L., Boisson, F., Fowler, S. W., & Fisher, N. S. (1996).  
27 Temperature effects on uptake and retention of contaminant radionuclides and trace  
28 metals by the brittle star *Ophiothrix fragilis*. *Marine Environmental Research*, 41,  
29 363-378.
- 30 Laporte, J. M., Truchot, J. P., Ribeyre, F., & Boudou, A. (1997). Combined effects of  
31 water pH and salinity on the bioaccumulation of inorganic mercury and

- 1       metheylmercury in the shore crab *Carcinus maenas*. *Marine Pollution Bulletin*, 34,  
2       880-893.
- 3       Lorenzo, J. I., Beiras, R., Mubiana, V. K., & Blust, R. (2005). Copper uptake by *Mytilus*  
4       *edulis* in the presence of humic acids. *Environmental Toxicology and Chemistry*, 24,  
5       973-980.
- 6       McLusky, D. S., Bryant, V., & Campbell, R. (1986). The effects of temperature and  
7       salinity on the toxicity of heavy metals to marine and estuarine invertebrates.  
8       *Oceanogr. Marine Biology Annual Reviews*, 24, 481-520.
- 9       Mubiana, V. K., Qadah, D., Meys, J., & Blust, R. (2005). Temporal and spatial trends in  
10       heavy metal concentrations in the marine mussel *Mytilus edulis* from the Western  
11       Scheldt estuary (The Netherlands). *Hydrobiologia*, 540, 167-180.
- 12       Mubiana, V. K., Vercauteren, K., & Blust, R. (in press) The influence of body size,  
13       condition index and tidal exposure on the variability in metal bioaccumulation in  
14       *Mytilus edulis*. *Environmental Pollution*.
- 15       Mubiana, V. K., Vercauteren, K., & Blust, R. (submitted). Effect of salinity and chemical  
16       speciation on the uptake of Cd, Co, Cu and Pb in marine mussels (*Mytilus edulis*).  
17       *Environmental Pollution*.
- 18       Newman, M. C. (1995). *Quantitative methods in aquatic ecotoxicology* (pp. 59-118).  
19       Boca Raton: CRC Press.
- 20       Phillips, D. J. H. (1976). The common mussel *Mytilus edulis* as an indicator of pollution  
21       by Zinc, Cadmium, Lead and Copper: effects of environmental variables on uptake of  
22       metals. *Marine Biology*, 38, 59-69.
- 23       Rainbow, P. S. (1997). Ecophysiology of trace metal uptake in crustaceans. *Estuarine*  
24       *Coastal Shelf Science*, 44, 169-175.
- 25       Rainbow, P. S. (2002). Trace metal concentrations in aquatic invertebrates: why and so  
26       what? *Environmental Pollution*, 120, 497-507.
- 27       Seed, R. & Suchanek, T. H. (1992). Population and community ecology of *Mytilus*. In E.  
28       Gosling (Ed.), *The Mussel Mytilus: Ecology, physiology, genetics and culture* (pp. 87-  
29       157). Amsterdam: Elsevier.

- 1 Smaal, A. S & Widdows, J. (1994). The scope for growth of bivalves as an integrated  
2 response parameter in biological monitoring. In K.J.M. Kramer (Ed.), *Biomonitoring*  
3 *of coastal waters and estuaries* (pp. 247-262). Boca Raton: CRC Press.
- 4 Sobral, P., & Widdows, J. (1997). Effects of elevated temperatures on the scope for  
5 growth and resistance to air exposure of the clam *Ruditapes decussates* (L), from  
6 Southern Portugal. *Scientia Marina*, *61*, 163-171.
- 7 Solorzano, L. (1969). Determination of ammonia in natural water by the phenol-  
8 hypochlorite method. *Limnology and Oceanography*, *14*, 799-801.
- 9 Simkiss, K., & Taylor, M. G. (1995). Transport of metals across membranes. In A.  
10 Tessier & Turner, D.R. (Eds.), *Metal Speciation and Bioavailability in Aquatic*  
11 *Systems* (pp. 1-44). John Wiley and Sons.
- 12 Vercauteren, K., & Blust, R. (1999). Uptake of Cd and Zn by the mussel *Mytilus edulis*  
13 and inhibition by calcium channel and metabolic blockers. *Marine Biology*, *135*,  
14 615-626.
- 15 Wang, J. F, Chuang, C. Y, & Wang, W-X. (2005). Metal and oxygen uptake in the green  
16 mussel *Perna viridis* under different metabolic conditions. *Environmental*  
17 *Toxicology and Chemistry*, *24*, 2657-2664.
- 18 Widdows, J. (1978). Physiological indices of stress in *Mytilus edulis*. *Journal of The*  
19 *Marine Biological Association of the United Kingdom*, *58*, 125-142.
- 20 Widdows, J., & Johnson, D. (1988). Physiological Energetics of *Mytilus edulis*: Scope  
21 for Growth. *Marine Ecology Progress Series*, *46*, 113-121.
- 22 Widdows, J. & Donkin, P. (1991). Role of physiological energetics in ecotoxicology.  
23 *Comparative Biochemistry and Physiology*, *100*, 69-75.
- 24 Widdows, J., Nasci, C., & Fossato, V. U. (1997). Effects of pollution on the scope for  
25 growth of mussels (*Mytilus galloprovincialis*) from the Venice Lagoon, Italy. *Marine*  
26 *Environmental Research*, *43*, 69-79.
- 27 Widdows, J., & Staff, F. (1997). *Practical procedures for the Measurement of Scope for*  
28 *growth*. Manual, Plymouth Marine Laboratory, Plymouth, U.K.
- 29 Widdows, J., Donkin, P., Staff, F., Matthiessen, P., Law, R. J., Allen, Y. T., Thain, J. E.,  
30 Allchin, C. R., & Jones, B. R. (2002). Measurement of stress effects (Scope for  
31 growth) and contaminant levels in mussels (*Mytilus edulis*) collected from the Irish  
32 Sea. *Marine Environmental Research*, *53*, 327-356.

1 Yang, H. N., & Chen, H. C. (1996). Uptake and elimination of cadmium by Japanese  
2 eel, *Anguilla japonica*, at various temperatures. *Bulletin of Environmental*  
3 *Contamination and Toxicology*, 56, 670-676.

4

## 5 **List of Tables and Figures**

### 6 **Table 1**

7 Mean ( $\pm$ SD) concentrations of dissolved metals ( $\mu\text{g/l}$ ) measured during 28 days of  
8 exposure. The table also shows average dissolved oxygen and pH over the same period.

### 9 **Table 2**

10 Summary of ANOVA for the effect of temperature on tissue metal concentrations before  
11 and after acclimation. The second and third columns show uptake slopes and elimination  
12 rate constants ( $k_e$  values), respectively. Marked \* denotes  $p < 0.05$ , <sup>ns</sup>  $p > 0.05$  and  
13 superscripts <sup>a, b & c</sup> refers to 6 °C, 16 °C and 26 °C, respectively.

### 14 **Table 3**

15 Kruskal-Wallis test results for differences among temperature treatments for the net  
16 uptake of Cd, Co, Cu and Pb in isolated gills of mussels.

17

### 18 **Figure 1**

19 Mean values ( $\pm$  SD) for soft tissue dry weight, maximum clearance rate, respiration rate  
20 and SFG of mussels. White bars represent field values, crossed for after acclimation  
21 period and shaded for end of the experiment. \* Marked values are significantly different  
22 ( $p < 0.05$ , Kruskal-Wallis with post-hoc) from a specific treatment denoted by the  
23 subscript.

### 24 **Figure 2**



1 Concentrations of metals ( $\mu\text{g/g}$  dry weight) in mussels as a function of time and fitted  
2 regressions (see text for details). Also shown are ANOVA results for effect of  
3 acclimation on metal concentration and results of *F*-statistic for effects of temperature  
4 on uptake slopes and elimination rate constants ( $k_e$  values).

5 **Figure 3**

6 Metal uptake in isolated gills and the effect of temperature on gills exposed to single  
7 metal (shaded bars) and to metal mixture (crossed bars). Indicated *p*-values are Kruskal-  
8 Wallis results comparing uptake in gills exposed to single and metal mixtures. Inserted  
9 graphs show net uptake in gills exposed to single metals (see Table 3 for statistical  
10 analysis).

11 **Figure 4**

12 The effect of temperature on metal speciation (percent activities) in chemically defined  
13 seawater with pH of 7.8 (at 6 °C), 7.9 (at 16 °C) and 8.0 (at 26 °C). Chemical speciation  
14 modelling was performed using the software Visual MINTEQ 2.32.

1

2

3

**Table 1**

4

Experimental temperature	Average dissolved metals in $\mu\text{g/l}$ ( $\pm$ SD)				[O <sub>2</sub> ]	pH
	<b>Cd</b>	<b>Co</b>	<b>Cu</b>	<b>Pb</b>	(mg/l)	
<b>6 °C</b>	3.3 (0.2)	2.9 (0.2)	4.0 (0.3)	2.2 (0.1)	10.4 (0.7)	7.8 (0.3)
<b>16 °C</b>	3.1 (0.1)	3.0 (0.2)	4.1 (0.3)	2.3 (0.2)	9.3 (0.8)	7.9 (0.2)
<b>26 °C</b>	3.2 (0.3)	2.9 (0.1)	4.2 (0.2)	2.3 (0.1)	7.4 (1.0)	8.0 (0.3)

5

ACCEPTED MANUSCRIPT

1  
2  
3

Table 2

	Effect of Acclimation Summary of ANOVA with post-hoc	Uptake rate Slopes ( $\pm$ SE) compared using <i>F</i> -statistic	Elimination $k_e$ ( $\pm$ SE) compared using <i>F</i> - statistic
<b>Cd</b>	Field vs 6°C *		
	Field vs 16°C *	<b>6°C</b> 0.219 (0.01)* <sup>b,c</sup>	No effect of temperature
	Field vs 26°C *	<b>16°C</b> 0.496 (0.04)* <sup>a,c</sup>	
	6°C vs 16°C <sup>ns</sup>	<b>26°C</b> 0.646 (0.02)* <sup>a,b</sup>	Pooled $k_e$ = 0.165 (0.026)
	6°C vs 26°C *		
	16°C vs 26°C *		
<b>Co</b>	Field vs 6°C <sup>ns</sup>	No effect of temperature	
	Field vs 16°C <sup>ns</sup>		
	Field vs 26°C *		
	6°C vs 16°C <sup>ns</sup>	Pooled slope = 0.046 (0.003)	Pooled $k_e$ = 0.096 (0.017)
	6°C vs 26°C <sup>ns</sup>		
	16°C vs 26°C *		
<b>Cu</b>	Field vs 6°C *		
	Field vs 16°C *	<b>6°C</b> 0.468 (0.026)* <sup>b,c</sup>	<b>6°C</b> 0.035 (0.01)* <sup>b,c</sup>
	Field vs 26°C *	<b>16°C</b> 0.140 (0.024)* <sup>a,c</sup>	<b>16°C</b> 0.129 (0.014)* <sup>a</sup>
	6°C vs 16°C *	<b>26°C</b> 0.049 (0.015)* <sup>a,b</sup>	<b>26°C</b> 0.115 (0.028)* <sup>a</sup>
	6°C vs 26°C *		
	16°C vs 26°C *		
<b>Pb</b>	Field vs 6°C *		
	Field vs 16°C <sup>ns</sup>	<b>6°C</b> 0.472 (0.029)* <sup>b,c</sup>	No effect of temperature
	Field vs 26°C *	<b>16°C</b> 1.001 (0.042)* <sup>a,c</sup>	
	6°C vs 16°C *	<b>26°C</b> 1.357 (0.064)* <sup>a,b</sup>	Pooled $k_e$ = 0.059 (0.010)
	6°C vs 26°C *		
	16°C vs 26°C *		

4

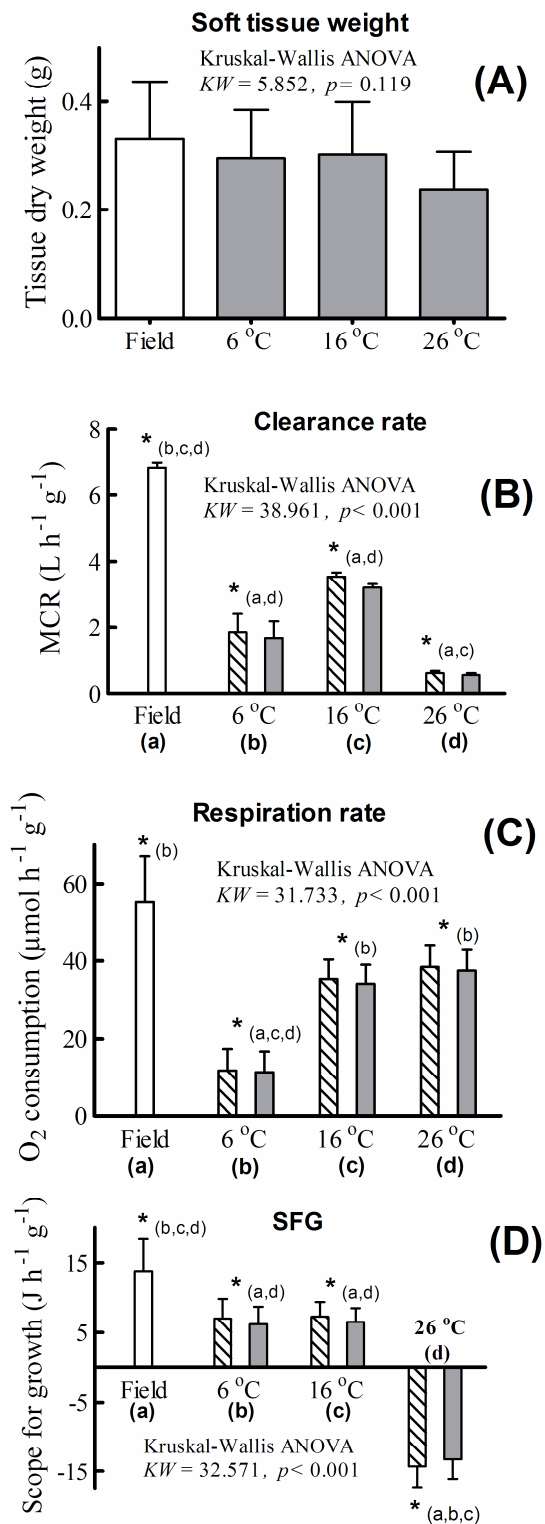
1  
2  
3  
4**Table 3**

Kruskal-Wallis test results			
	<i>KW statistic</i>	<i>p-level</i>	<i>(n)</i>
<b>Cd</b>	15.283	0.0005	(42)
<b>Co</b>	12.737	0.0017	(43)
<b>Cu</b>	7.752	0.0207	(42)
<b>Pb</b>	11.673	0.0029	(42)

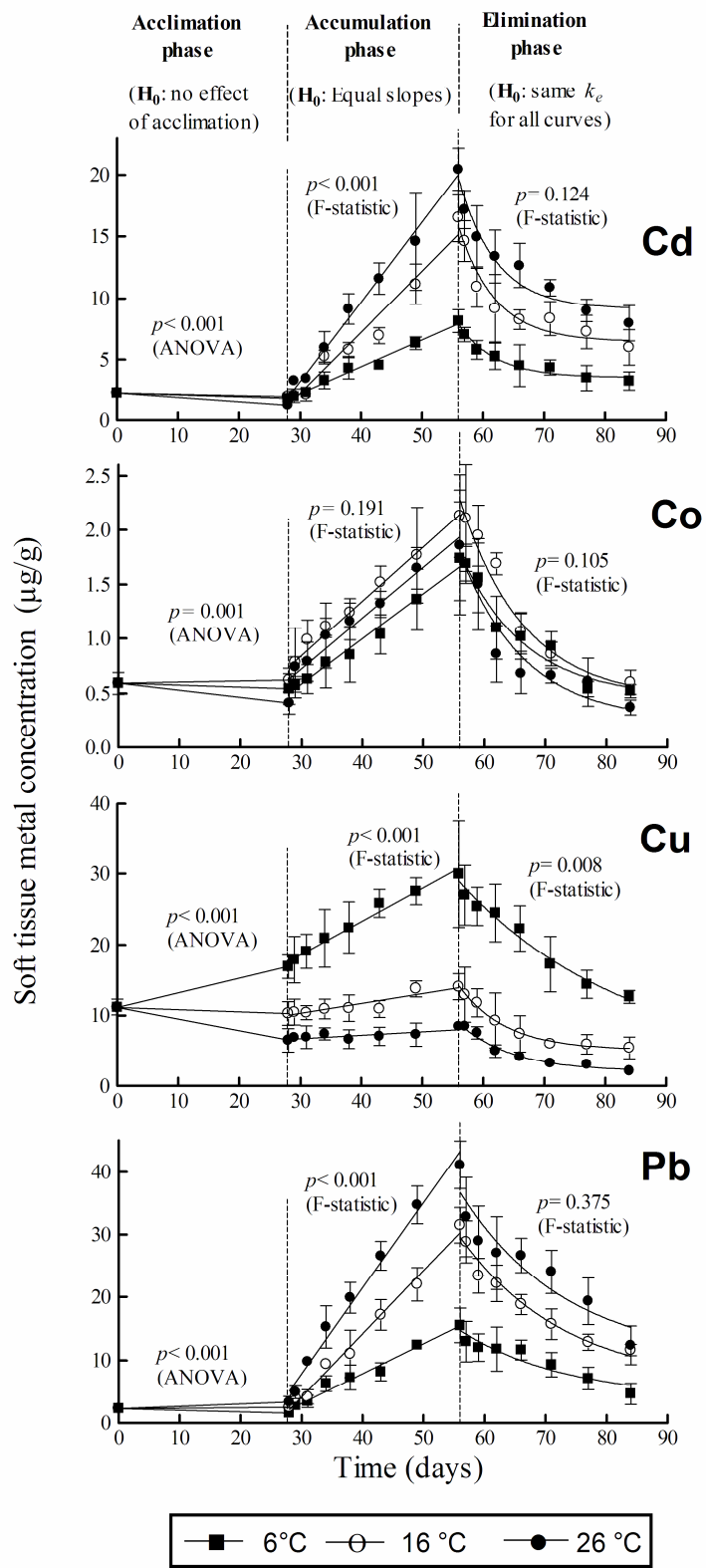
5

ACCEPTED MANUSCRIPT

1 Figure 1

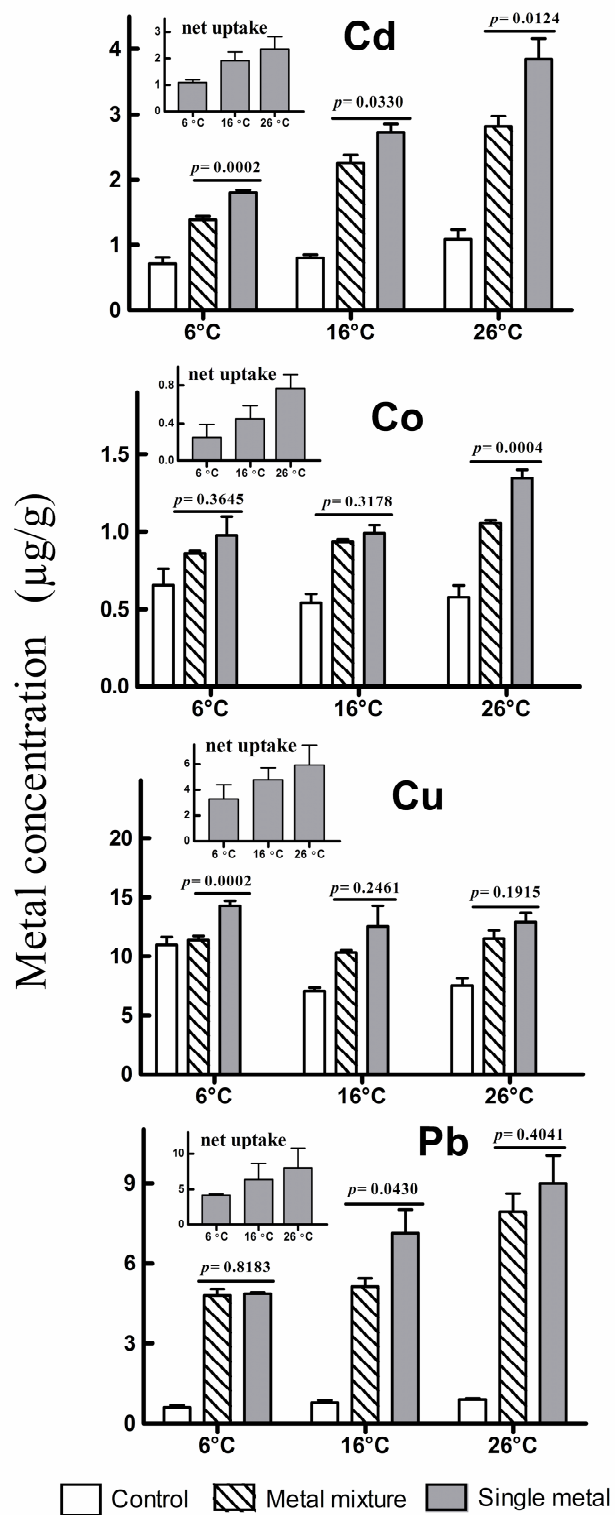


1 Figure 2



2

1 Figure 3



2

3

1 Figure 4

