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Effects of temperature on scope for growth and accumulation of Cd, Co, Cu and Pb by the marine bivalve *Mytilus edulis*

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Abstract

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

Keywords: Mussels; Heavy metals; Bioaccumulation; Temperature; Speciation; Scope for growth

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1. Introduction

Bioaccumulation of heavy metals in aquatic organisms such as mussels depends not only on environmental concentrations but also on a variety of biological and environmental factors. The common marine mussel *M. edulis* is widely used in monitoring metal pollution in coastal and estuarine waters (e.g. Goldberg et al., 1983; De Kock and Kramer, 1994). Therefore, understanding the possible roles of temperature and other environmental factors on metal uptake and accumulation in these biomonitors is critical in order to correctly relate tissue concentrations to those in the surrounding environment. Temperature affects both metal chemistry in seawater (Byrne et al., 1988) and physiology of mussels (Dame, 1996). Temperature affects metal chemistry by changing chemical speciation, pH, solubility, reaction rates or physical kinetics (Byrne et al., 1988; Blust et al., 1994). Theoretical calculations of chemical speciation in seawater indicate that changes in temperature and pH have most effects on strongly hydrolysed and carbonate complexes and less effect on chlorides and free metal ions (Byrne et al., 1988). Though it is widely known that uptake is largely controlled by the free metal ion, some studies have also shown significant contribution of other species, particularly metals bound to weak complexes (i.e. Campbell, 1995; Hudson, 1998; Lorenzo et al., 2005). Chemical speciation indicates that increase in temperature generally results in increase in the concentrations and activities of bioavailable metal forms, therefore, enhance uptake, at least in theory.

Like most aquatic organisms, mussels cannot regulate their body temperature in accordance with surrounding environment, as a result most physiological and biochemical processes are temperature dependent (Dame, 1996). One measure of animal's integrated physiological status is Scope for growth (SFG), which is essentially an energy balance equation of an individual, determined from energy absorbed from ingested food items minus the energy lost through respiration and excretion (Widdows, 1978; Widdows and Johnson, 1988). During the past 15 years, many studies have applied SFG approach as a quantitative measure of the impact of environmental stress due to i.e. salinity and temperature (Widdows, 1978; Sobral and Widdows, 1997; Smaal and Widdows, 1994; Vercauteren and Blust, 1999) and chemical pollution (Widdows and Johnson, 1988; Widdows et al., 1997, 2002; Smaal and Widdows, 1994). The role of physiological energetics such as SFG in ecotoxicology is discussed in detail in a review by Widdows and Donkin (1991). Smaal and Widdows (1994) outlined the justification for use of SFG as an integrated response in biological monitoring programmes.

The role of temperature on metal uptake and accumulation in marine mussels has not been well studied. This is despite the fact that mussels are important biomonitoring organisms. Furthermore, there seem to be no general consensus among the few available studies on the subject. Carpene and George (1981) found no temperature effects on Cd uptake in isolated gills. However, lack of prior temperature acclimation of mussels used in that study may have influenced the results. In whole organism experiments, Phillips (1976) detected no temperature effects on accumulation of Cd, Cu, Pb and Zn except at low salinity (<18 ppt). Fischer (1986) also observed that Cd accumulation by mussels was only significantly decreased at very low temperatures (<7 °C). In both studies temperature exposures were coupled with different salinity treatments, therefore it is not clear whether salinity or interaction between the two factors may have influenced the results. Some recent studies have reported uptake that is dependent and increases with increase in temperature (Wang et al., 2005). Baines et al. (2005) also found a positive relationship between assimilation

efficiency of dietary metals with temperature. These studies are consistent with results involving many different organisms, which tend to show a positive correlation between temperature and metal uptake, accumulation or toxicity (McLusky et al., 1986; Blust et al., 1994; Bervoets et al., 1996; Chowdhury and Blust, 2001).

The objective of the current study was to investigate the influence of temperature on uptake and accumulation of two types of metals i.e. Co, Cu as biologically essential metals and Cd and Pb as non-essential metals. Accumulation kinetics of these metals were determined in long-term exposure experiments. In addition, short-term exposures involving isolated gills were also conducted. These experiments involved exposure of gills to either single metal solutions or metal mixtures. The two treatments were intended to check whether there were significant interactions among metals during uptake. Isolated gills experiments minimised the influence of animal physiology and hence provide a better insight into the fundamental mechanisms and processes involved. The main consequences of temperature changes in the exposure water namely, metal speciation, pH and dissolved oxygen were also determined. SFG was determined as an integrated measure of the animal physiological status in response to differences in ambient temperature.

2. Materials and methods

2.1. Test animals

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

2.2. Exposure medium

Artificial seawater was prepared by dissolving 35 g/l *hw Sea Salt professional* (Wiegandt GmbH, Germany) in deionised water. Elemental composition of this salt is well described and contains only traces of heavy metals i.e. Cu (200 ng/kg), Pb (19 ng/kg), Co (3 ng/kg) and Cd (0.1 ng/kg) (http://www.hw-wiegandt.de). Metal exposure solutions were then prepared by dissolving high purity salts of $CdCl_2$, $CoCl_2$, $CuCl_2$ and $PbNO_3$ either as a mixture of all four metals or as single metal solutions. The exposure concentrations used (see Table 1) were neither too low for measurable uptake nor too high to be environmentally relevant. However, in view of the short exposure times for isolated gills (1 h), relatively high exposure concentrations (0.5 μM) were used in order to achieve measurable uptake in the gills.

Table 1
Mean (±SD) concentrations of dissolved metals (μg/l) measured during 28 days of exposure

Experimental temperature (°C)	Average dissolved metals in μg/l (±SD)				$[O_2]$ (mg/l)	pH
	Cd	Co	Cu	Pb		
6	3.3 (0.2)	2.9 (0.2)	4.0 (0.3)	2.2 (0.1)	10.4 (0.7)	7.8 (0.3)
16	3.1 (0.1)	3.0 (0.2)	4.1 (0.3)	2.3 (0.2)	9.3 (0.8)	7.9 (0.2)
26	3.2 (0.3)	2.9 (0.1)	4.2 (0.2)	2.3 (0.1)	7.4 (1.0)	8.0 (0.3)

The table also shows average dissolved oxygen and pH over the same period.

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

2.3. Whole organism experiments

After sampling, animals were transported on ice to the laboratory and on arrival 20 and 14 individuals were chosen randomly for metal analysis and SFG measurements, respectively. Remaining mussels were divided into three groups of about 250 mussels and each group was placed in 500 l tanks. Initially, temperature was set at 13 °C (field temperature) then after a week and during two weeks it was slowly adjusted to obtain 6 °C, 16 °C and 26 °C after which acclimation was then continued for another week. Mussels were always fed 10 mg /individual of enriched yeast cells (Lanzy PZ, Inve, Belgium) twice a week. To minimise organic matter loading in the experimental tanks, feeding of mussels was in separate tanks during 3 h with conditions identical to experimental tanks. Furthermore, organic carbon was monitored every 3 days using a TOC 5000 Analyzer (Shimadzu) fitted with a 0.8 mm diameter suspended particle sampler. Both DOC and POC varied insignificantly with values consistently below 0.1 mg/l and 0.01 mg/l, respectively.

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

Five ml high purity concentrated HNO_3 and 0.25 ml of 27% H_2O_2 were then added and the digest was further destructed by microwave heating according to Blust et al. (1988). Samples were heated in four steps of 5 min at 80, 160, 240 and 320 W, and then diluted with purified water to bring acid concentration to about 2%. Metal concentrations in the final solutions were measured with ICP-MS (Ultra Mass 700, Varian Australia) employing yttrium and indium as internal standards. As quality control, three reference samples (mussel tissue CRM 278R, IRMM, Geel Belgium) and three preparation blanks were included in each 40-sample rack and were carried along in every step of the analysis. Recoveries for all four metals in the reference samples were consistently within 96–101% of the certified values.

2.4. Measurements of scope for growth

Scope for growth was measured after field sampling (day 0), after temperature acclimation (day 21) and at the end of experiment (day 84). The measurements were conducted according

to a procedure adopted from Widdows and Johnson (1988) and Widdows and Staff (1997). Unless stated, all the constants used in the equations were adopted from these references. The first SFG parameter determined was clearance rate (CR). Mussels were placed in clean filtered seawater for 1 h to flush-out any particles enclosed inside shell valves and then individually placed in 2 l seawater, which was previously filtered (0.45 μm) and then spiked with yeast cells (Lanzy PZ, Inve, Belgium) at 50,000 cells/ml. The vessels were aerated to provide homogenous distribution of cells and then every 15 min for 90 min, 10 ml water samples were taken for cell counting with a Coulter Counter. By definition, CR is the volume of water cleared of particles per gram mussel per hour and was calculated according to the expression:

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

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

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

where, POM is concentration of yeast cells (mg/l) with energy of 23 J mg^{-1} ash free dry weight (obtained from the manufacturer, Inve, Belgium) and 0.5 is absorption efficiency from yeast cells, which was calculated from ash free dry weight of ingested cells and faeces produced by the mussels.

After measuring CR, each mussel was then transferred into a 500-ml respirometer filled with clean filtered (0.45 μm) water and fitted with an oxygen electrode connected to a computer, which recorded dissolved oxygen over time (90 min). Measured respiration rates were then converted into energy equivalent according to the formula:

$$\text{Energy respired (J/h/g)} = \text{Respiration rate } (\mu\ \mathrm{moles\ O_2\ g^{-1}\ h^{-1}}) \times 0.456$$

where 0.456 J $\mu mole^{-1}$ O_2 is the heat equivalent of oxygen uptake.

Energy excreted was calculated from ammonia produced during respiration measurements. Water samples were taken before and after incubation and were filtered (0.45 μm) and then analysed for ammonia concentration according to the phenol-hypochlorite method of Solorzano (1969). Ammonia produced by each mussel was then converted into energy equivalents as follows:

$$\text{Energy excreted (J/h/g)} = \text{Ammonia excretion rate } (\mu\ \mathrm{moles\ NH_3\text{-}N\ g^{-1}\ h^{-1}}) \times 0.349$$

where excretion of 1 μmole NH_3-N h^{-1} is equivalent to energy loss of 0.349 J h^{-1}.

Finally SFG was calculated as:

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

2.5. Metal uptake in isolated gills

For each temperature treatment, three groups of gills consisting of 14–15 replicates were obtained from fully temperature acclimated mussels. After extraction, gills from one group were rinsed in clean seawater and individually incubated for 1 h in 300 ml of

clean seawater (controls). The second and third groups were incubated under the same conditions and at 0.5 μM single and metal mixture solutions, respectively.

After incubation gills were washed three times in 300 ml clean seawater and then in purified water. This procedure was found to be sufficient to remove loosely adsorbed metals. It is generally accepted that strongly adsorbed metals are associated more with internalised than external metal fraction (Endo et al., 1998). After washing gills were placed in pre-weighed polypropylene tubes and freeze –dried at −60 °C during 72 h. Dry samples were then digested in 1 ml HNO_3 and 0.05 ml H_2O_2 as described earlier and reference material and preparation blanks were also included. Metal concentrations in diluted solutions were then analysed with ICP-MS as earlier described.

2.6. Modelling bioaccumulation

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

$$C_{\text{mussel}} = a + b * e^{-k_e t}$$

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

3. Results

3.1. Scope for growth

Fig. 1a shows soft tissue dry weights (mean of 14–20 mussels) at the time of sampling (field) and at the end of the experiment. Since sample sizes were small data were tested for differences using Kruskal–Wallis ANOVA and results showed no significant differences among the four groups. Fig. 1b and c shows MCR and respiration rates of mussels after sampling (field) and after acclimation (crossed bars) and at end of experiment (grey bars). Both data were subjected to Kruskal–Wallis test with Dunn's post-hoc and results showed higher MCR under field conditions and there were significant differences among temperature treatments (as shown in the Figures). Respiration rate was only significantly lower at 6 °C. Both respiration rate and MCR showed no differences between measurements performed after acclimation and after metal exposures (results not shown for clarity), similar trend was shown for energy excreted (as ammonia) but there were differences among temperature treatments with highest energy excreted at 26 °C (1.26 ± 0.2 J/h/g) followed by 16 °C (0.23 ± 0.05 J/h/g), then field (0.13 ± 0.03 J/h/g) and the least was at 6 °C (0.05 ± 0.01 J/h/g). Energy excreted contributed less 1% towards SFG while clearance rate (or food acquisition) contributed most, consistent with literature (Sobral and Widdows, 1997; Widdows and Staff, 1997). Finally, as shown in Fig. 1d, SFG was highest under field conditions, lowest at 26 °C and there was no difference between 6 °C and 16 °C.

3.2. Metal accumulation in whole organisms

Fig. 2 shows metal concentrations in mussels at sampling (day 0), during metal exposure (day 28–56) and during depuration (day 56–84). In order to determine

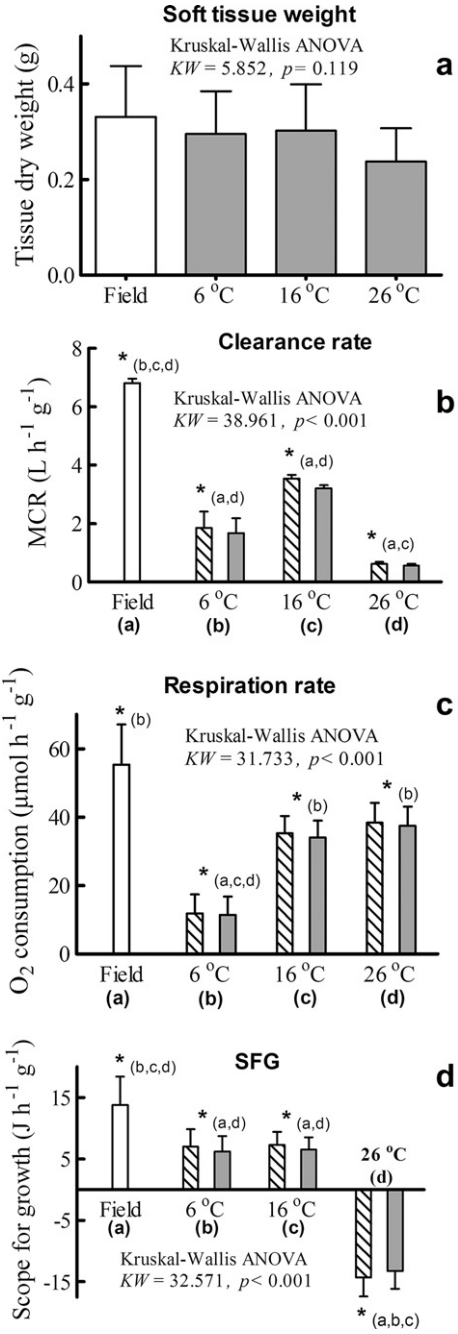

Fig. 1. Mean values (±SD) for soft tissue dry weight, maximum clearance rate, respiration rate and SFG of mussels. White bars represent field values, crossed for after acclimation period and shaded for end of the experiment. *Marked values are significantly different ($p < 0.05$, Kruskal–Wallis with post-hoc) from a specific treatment denoted by the subscript.

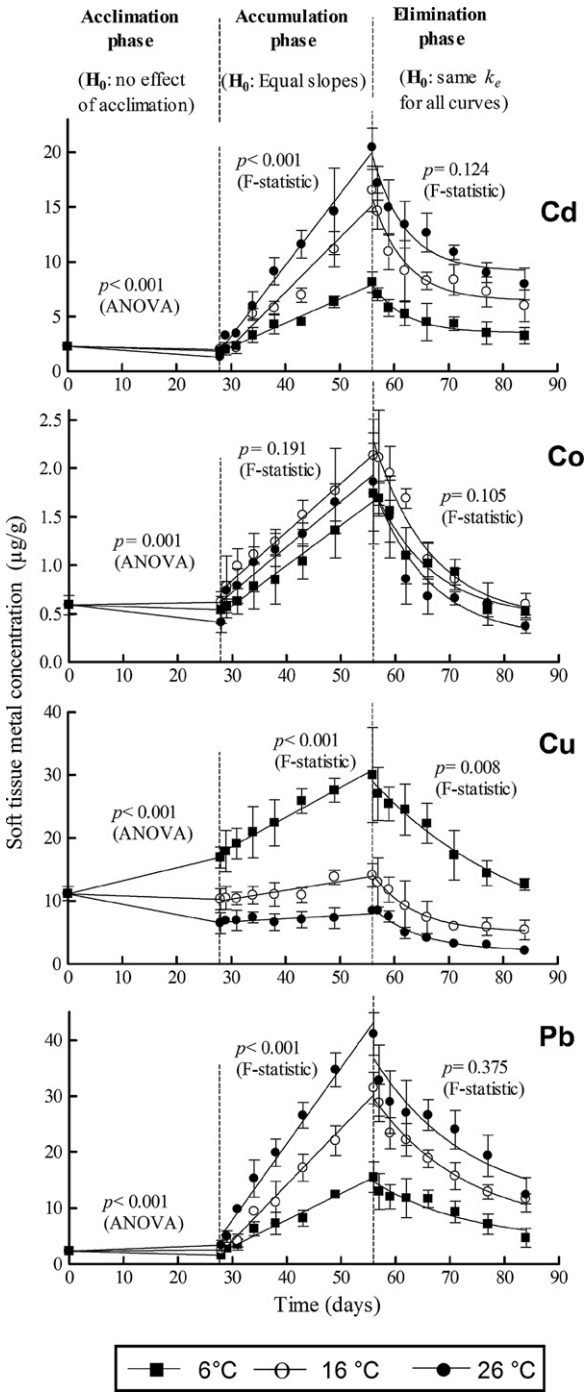

Fig. 2. Concentrations of metals (µg/g dry weight) in mussels as a function of time and fitted regressions (see text for details). Also shown are ANOVA results for effect of acclimation on metal concentration and results of *F-statistic* for effects of temperature on uptake slopes and elimination rate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

whether acclimation and temperature had effect on metal concentrations, day 0 and day 28 data in all three temperature treatments were subjected to ANOVA. Results showed that overall there were significant differences ($p < 0.05$) as shown in the figure and post-hoc test results for specific differences are summarized in Table 2. Metal accumulation increased linearly with time and in order to evaluate the effect of temperature, slopes of the linear lines were compared using *F-statistics*. Results showed significant differences except for Co concentration whose pooled slope was calculated as 0.046. Post-hoc test (Table 2) showed that slopes increased with increase in temperature for the non-essential metals Cd and Pb while the slope for Cu decreased with temperature.

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

Table 2
Summary of ANOVA for the effect of temperature on tissue metal concentrations before and after acclimation

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

The second and third columns show uptake slopes and elimination rate constants (k_e values), respectively. Marked * denotes $p < 0.05$, $^{ns}p > 0.05$ and superscripts ^{a,b,c} refers to 6 °C, 16 °C and 26 °C, respectively.

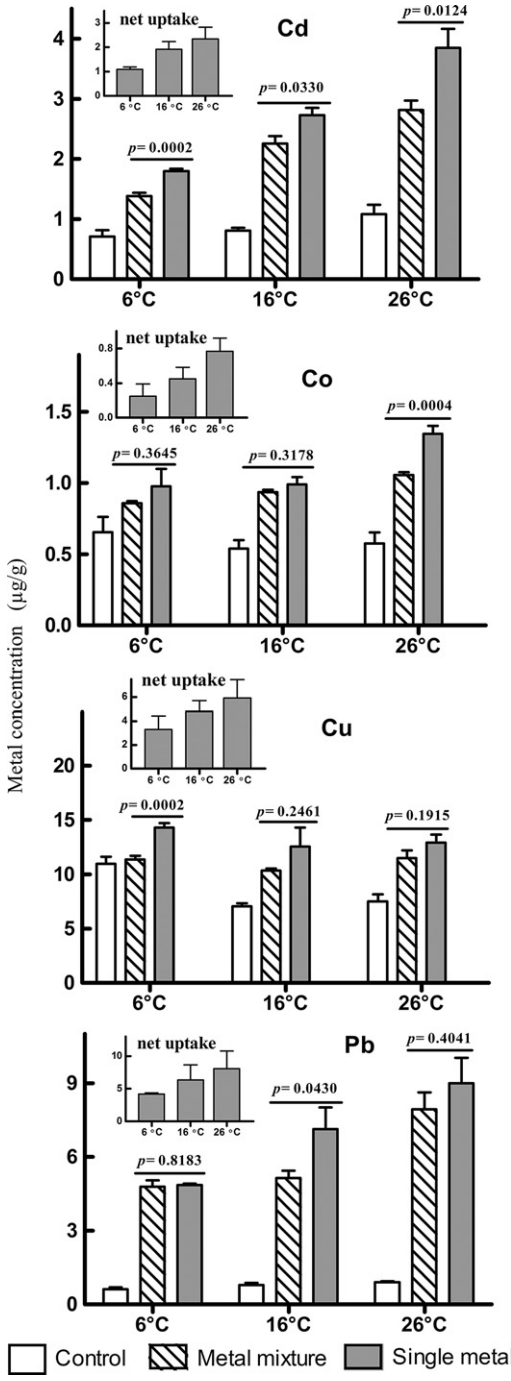

Fig. 3. Metal uptake in isolated gills and the effect of temperature on gills exposed to single metal (shaded bars) and to metal mixture (crossed bars). Indicated *p*-values are Kruskal–Wallis results comparing uptake in gills exposed to single and metal mixtures. Inserted graphs show net uptake in gills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

Table 3
Kruskal–Wallis test results for differences among temperature treatments for the net uptake of Cd, Co, Cu and Pb in isolated gills of mussels

	Kruskal–Wallis test results		
	KW statistic	*p*-level	(*n*)
Cd	15.283	0.0005	(42)
Co	12.737	0.0017	(43)
Cu	7.752	0.0207	(42)
Pb	11.673	0.0029	(42)

3.3. Metal uptake in isolated gills

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

Inserted graphs in Fig. 3 show metal uptakes in gills exposed to single metal solutions. The values were calculated as averages (±SD) of the differences between mean controls and concentration in individual gills. The data were subjected to Kruskal–Wallis ANOVA and results as shown in Table 3 indicated significant differences, confirming that uptake was higher at higher temperature.

4. Discussion

The study showed that with exception of Cu, tissue concentrations decreased during acclimation as was expected due to low background levels of metals in the acclimation water. However, the decreases appeared to depend on temperature of acclimation, with animals in the lowest temperature losing most metals. This effect was in contrast with later results during elimination phase where no effects of temperature were observed with the exception of copper (Fig. 2 and Table 2). Therefore, temperature-effects during acclimation appeared to be an initial response, which disappeared once animals were fully acclimated and had been exposed to higher metal concentrations. It is not clear what kinds of processes are responsible for such responses. However, it is reasonable to assume that some physiological and biochemical changes related to temperature acclimation may be somehow responsible for influencing metal loss during the early stages of acclimation. These types of effects are relevant for inter-tidal organisms such as mussels, which are often exposed to temperature fluctuations during tidal cycle.

The general lack of temperature effects on elimination especially for non-essential metals Cd and Pb is consistent with some studies on marine bivalves (Amiard et al., 1986, 1987; Hutchins et al., 1998), echinoderms (Hutchins et al., 1996) and Japanese eel (Yang and Chen, 1996). In field studies seasonal variations in tissue metal concentrations may to some extent imply temperature effects. However, some studies seem to indicate that most

of the seasonal variability may well be due to changes in animal body weight, since total body burdens remained largely unchanged (Cossa et al., 1979; Amiard et al., 1986; Mubiana et al., 2005). However, size related factors (i.e. growth rate, age and body size), can still significantly influence not only concentrations but can also affect bioaccumulation of metals in marine mussels (Mubiana et al., 2006). Therefore, changes in body weight during long-term exposure experiments can have an influence on accumulated metals. However, in this particular study, differences in tissues weights among the treatments before and after exposures were not significantly different (Fig. 1a). Therefore, change in tissue weight could not have been a significant factor responsible for the observed metal accumulation patterns, other factors must be evoked.

As demonstrated in this study and evidence from literature, it seems more likely that in the long-term, temperature has little or no influence on elimination of most metals, except perhaps for some essential metals such as Cu. In the case of Co, the lack of temperature-effects on both accumulation and elimination in whole organisms despite clear temperature effects at isolated gills level is further indication of physiological regulation. It is well known that most organisms are capable of controlling internal concentrations of essential metals up to a certain threshold level (Rainbow, 1997, 2002). Animals regulate metal concentrations either in response to external metals exposures and /or in response to environmental factors including temperature or due to attainment of critical body levels (Rainbow, 2002).

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

In seawater, temperature influences a variety of physiochemical factors and the important ones include changes in pH and chemical speciation of metals (Byrne et al., 1988). As shown in Fig. 4 and consistent with literature, temperature showed little effect on metal speciation, particularly on the free metal ion activity, which is the most bioavailable form for most metals and organisms (Campbell, 1995; Hudson, 1998). Generally, increase in temperature results in the increase in free metal ion activity and a few other metal species like carbonates. Some studies have indicated that besides free metal ions, other metal forms such as OH^- and CO_3 complexes can somehow be bioavailable to organisms (Hudson, 1998; Laporte et al., 1997). Therefore, although temperature-effects on metal speciation were generally modest at least within environmentally relevant temperature ranges, nevertheless, it was expected that increase in temperature would generally favour higher uptake. Besides speciation effects, changes of most physiochemical factors such as chemical reaction and diffusion rates would favour higher uptake with increase in temperature

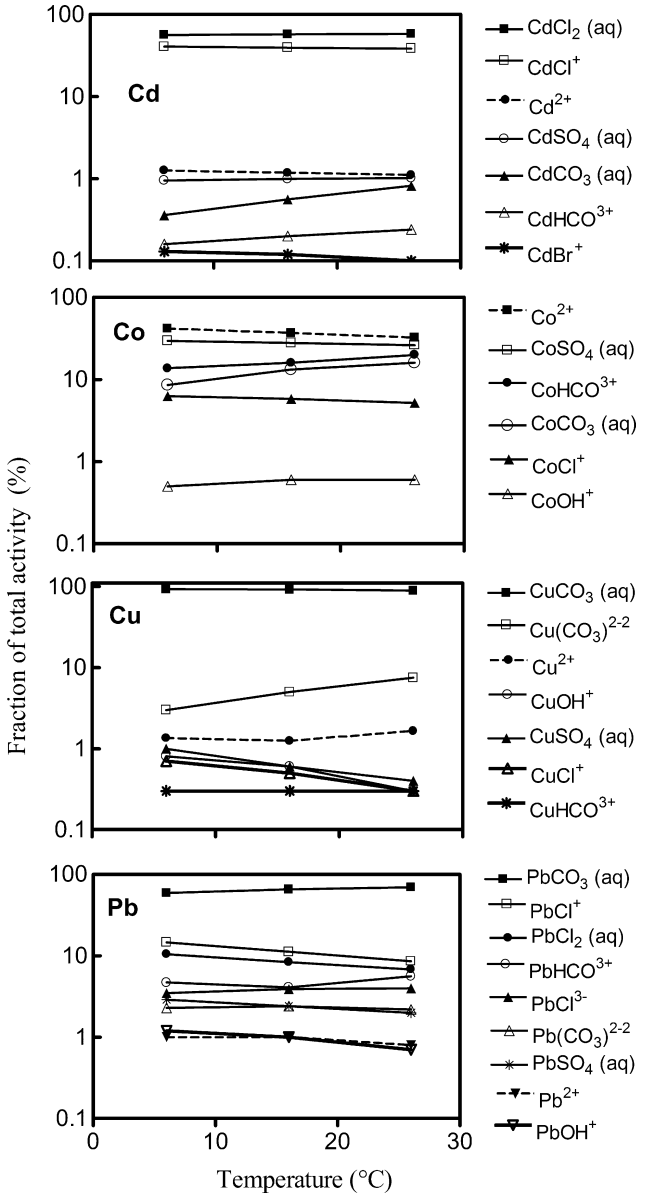

Fig. 4. The effect of temperature on metal speciation (percent activities) in chemically defined seawater with pH of 7.8 (at 6 °C), 7.9 (at 16 °C) and 8.0 (at 26 °C). Chemical speciation modelling was performed using the software Visual MINTEQ 2.32.

(McLusky et al., 1986; Blust et al., 1994; Bervoets et al., 1996; Chowdhury and Blust, 2001). In the case of pH, since it is usually positively correlated with temperature (Byrne et al., 1988), increase in temperature was expected to decrease metal uptake. However, since seawater has high buffering capacity, only moderate changes in pH between

6–26 °C were observed (Table 1). On this basis pH was not expected to be a major factor determining metal uptake or accumulation in this study.

The range of SFG determined in this study is comparable with literature concerning *M. edulis*. As shown in Fig. 1, clearance rate of about $7\,l\,g^{-1}\,h^{-1}$ corresponding to SFG of nearly 14 J/g/h measured under field conditions, which are within a typical range for mussels from clean environment (i.e. Widdows and Staff, 1997; Smaal and Widdows, 1994). SFG measurements representing field conditions (day 0) are consistent with the fact that Eastern Scheldt is well known for its favourable conditions for shellfish farming in The Netherlands. However, after laboratory acclimation SFG decreased almost by half at 6 °C and 16 °C. This decrease was mainly due to low clearance rate, which is not surprising considering the obvious differences between field and laboratory conditions in terms of food quality and quality. Negative SFG at 26 °C implied that animals were utilizing energy reserves, mainly because high temperature seriously inhibited clearance rate. As demonstrated in other studies (i.e. Sobral and Widdows, 1997; Smaal and Widdows, 1994) clearance rate (or food acquisition) is the most sensitive parameter for SFG. The study showed that apart from the initial drop in SFG during acclimation, temperature only influenced SFG at the highest temperature while there was no significant difference between 6 and 16 °C. This is consistent with the fact that temperate *Mytilus spp.* are well known for their ability to tolerate very low temperatures (<10 °C) whereas very high temperatures (generally >22 °C) can have very serious detrimental effects on mussels (Seed and Suchanek, 1992; Dame, 1996). In this respect, the study has shown that in terms of energy budgets, mussels are physiologically more tolerant to very low temperatures. The study has also shown that SFG can be a useful tool to determine stress from high temperature exposures such as occasionally experienced in the summer periods.

SFG is essentially an integration of many complex physiological processes some of which can be directly or indirectly linked to metal uptake or accumulation in mussels. For example, change in clearance rate has the potential to affect exposure to dissolved metals, but the nature of the relationship is not yet clear. Theoretically, increased clearance rate implies increased contact with waterborne metals and thus enhanced uptake and accumulation. However, Blackmore and Wang (2003) observed low uptake among mussels with high filtration rates, though those results may have been influenced by other factors. Respiration rate also has the potential to affect metal content of mussels, Wang et al. (2005) found uptake of Cd and Zn to decrease with decrease in respiration rate. In this study, none of the SFG parameters could be directly correlated with observed metal uptake or accumulation among the temperature treatments. This does not in anyway exclude effects of SFG on metal accumulation, what the results highlight is the fact that temperature related physiological responses are complex and many of them can directly or indirectly influence metal uptake and accumulation. Further research is required especially including wide differences in terms of SFG, in order to better assess the impact or lack of SFG on metal accumulation in mussels.

The study has shown that when considered at the solution–organism interface such as isolated gills, temperature effects on both essential and non-essential metals is largely controlled by solution chemistry and physical kinetics, which tend to favour higher uptake with increase in temperature (Byrne et al., 1988; Blust et al., 1994; Simkiss and Taylor, 1995). However, at whole organism level, complex physiological responses tend to mask the relationship, particularly for biologically essential metals like copper, which are subject

to regulation over a certain range and conditions (Rainbow, 2002; Amiard et al., 1986, 1987).

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