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The influence of body size, condition index and tidal exposure on the variability in metal bioaccumulation in *Mytilus edulis*

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Body size, condition index and shore height can modify metal concentrations in mussels and if not taken into account, can lead to wrong interpretation of monitoring data.

Abstract

Mussels are commonly used to monitor metal pollution despite high inter-individual variability in tissue concentrations. In this study, influences of body size, condition index and tidal height on concentrations of As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn were investigated. Body weight was inversely related to metal concentrations and for Cd, Mn, Pb and Zn the regression was affected by tidal height. Except for As, Fe and Mn metal concentrations were inversely related to physiological status though no differences between essential and non-essential metals were obvious. After correcting for body size, tidal height was related positively to As, Cd and Zn, negatively related to Cu, Fe and Mn while Co, Cr, Ni and Pb were independent of tidal height. The study recommends stringent measures during sampling for biomonitoring or metal concentrations at each location must be normalized to a common body size, CI and tidal height.

Keywords: Mussels; Heavy metals; Inter-individual variability; Biomonitoring

1. Introduction

Since the early works of Goldberg (1975) and Phillips (1976), marine mussels have been used as model organisms to study and monitor environmental metal pollution in coastal waters world-wide (e.g. Goldberg et al., 1983; Luten et al., 1986; Stronkhorst, 1992; De Kock and Kramer, 1994). In biomonitoring programmes, the primary objective is to relate metal body contents of mussels to bioavailable levels found in the surrounding environment. It is well known that mussels are good accumulators of heavy metals and generally body concentrations tend to reflect environmental exposures. However, despite the numerous studies on mussels, quantitative extrapolation of environmental exposures from metal body concentrations or vice versa, still remain with lots of uncertainty (Rainbow, 2002). This is mainly due to several biological and environmental factors that influence accumulation and concentrations of metals in mussel tissues. Therefore, a clear understanding of these factors is critical in order to establish quantitative relationships relating body concentrations to external exposures.

Phillips (1976) recognised the potential effects of environmental variables on the accumulation of metals in mussels. Since then, influence of many factors have been studied such as salinity, season and organic matter (De Kock and Kramer, 1994; Fisher et al., 1996; Vercauteren and Blust, 1996), body size (Boyden, 1974; Riget et al., 1996; Wang and Fisher, 1997), sex and reproductive status (Lobel et al., 1991; Widdows and Donkin, 1992), tidal height (Phillips, 1976; Lobel and Wright, 1982) and physiological condition (Lobel et al., 1991; Widdows and Donkin, 1992). However, effects of these factors in quantitative terms still remain unclear with different results reported depending on the study, element, location or season (Riget et al., 1996; Rainbow, 2002). Therefore, one of the objectives of this study was to contribute

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towards understanding some of the factors responsible for causing such variations.

When studying mussels collected from different habitats or season, variations in the average body size, animal condition and vertical position are among the most predictable variables. From available literature, different patterns have been reported relating body size to tissue metal concentrations in mussels (Boyden, 1974; Riget et al., 1996). Boyden (1974) reviewed relationships between body size and tissue concentrations of different metals in different molluscs. It was shown that metal concentrations in molluscs can either be positively, negatively correlated or independent of body size. However, later studies have reported results that are either consistent or contrary to the list provided by Boyden (1974) (Lobel and Wright, 1982; Riget et al., 1996).

The relationship between body weight and metal concentration is often described using power functions or their logarithmic transformations (Boyden, 1974). However, within a narrow range of body size, linear equations are more appropriate (Newman, 1995). Studies have shown that the regression slopes relating tissue metal concentrations to body size are not constant but may vary significantly depending on location, habitat or year (Lobel and Wright, 1982; Riget et al., 1996). However, the factors causing such variations are still unknown (Riget et al., 1996; Wang and Fisher, 1997). In the current study the influence of tidal exposure (represented by shore height or distance from the low tide mark) was examined for several heavy metals to detect general bioaccumulation patterns.

The main objective of the study was to assess the significance of three factors, body size, animal condition and tidal exposure toward natural high inter-individual variability in the tissue concentrations of heavy metals in mussels. Since previous studies focused on one or two metals (e.g. Zn – Lobel and Wright, 1982), the present study aimed to include a wide range of metals (As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in order to gain insight into general patterns, particularly to contrast between biologically essential metals and non-essential ones.

2. Materials and methods

2.1. Sampling

The study site (Westkapelle) is located on the Dutch coast close to the mouth of the Scheldt Estuary (Fig. 1). The mussel bed stretches for approximately 3 m between the low water mark and the uppermost distribution of the mussels at the site. The site was chosen because it represented moderate contamination of heavy metals in mussels (Mubiana et al., 2005).

Mussels were collected from seven shore (or tidal) height intervals starting from the low tide mark (0 m) to the highest point (2.8 m) of mussel distribution. At each height interval 40–60 individual mussels of different sizes were harvested. For each individual shell dimensions, length, width and height were measured to the nearest 0.1 mm with Vernier callipers. The volume of each individual was also determined by the water displacement method to the nearest 0.1 or 0.5 cm^3, depending on the size of the individual and after dissection, volume of empty shells was also determined in the same way.

2.2. Metal determinations

After dissection, the soft tissues were blotted with tissue paper and wet weight of each individual was determined. The tissues were then placed in pre-weighed 50-ml polypropylene tubes and dried at 60 °C during 72 h after which the tubes were reweighed and the dry weight was calculated. Five millilitres concentrated HNO_3 and 0.25 ml high purity H_2O_2 were then added. Three blank samples and three reference samples of mussel tissues (BCR 278R,

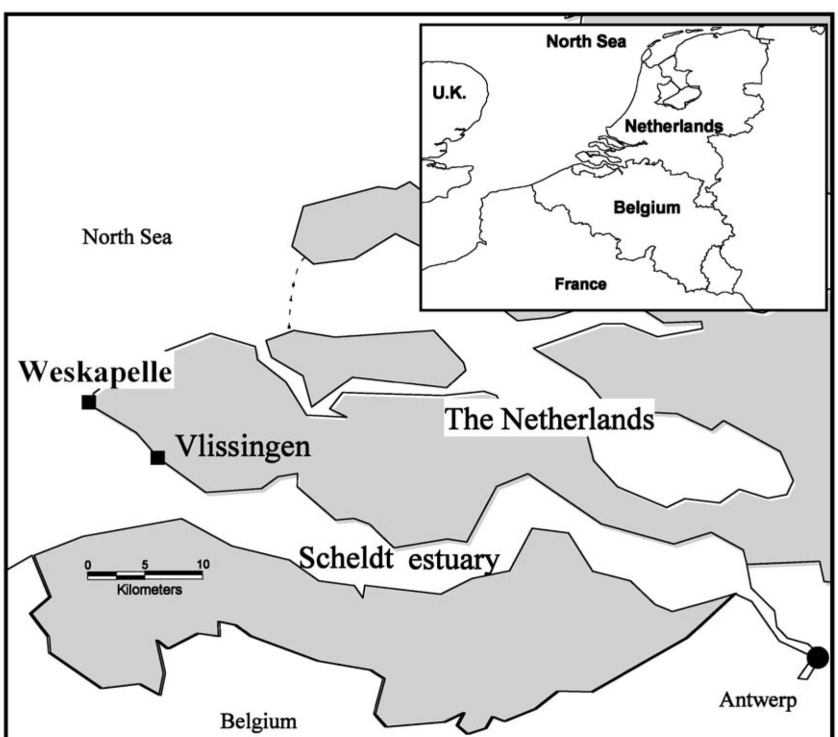

Fig. 1. Map showing the sampling site of Westkapelle on the Dutch coast (The Netherlands).

Institute of Reference Materials and Measurements, European Commission, Geel, Belgium) were included for every 34 samples. Generally, recoveries for these reference samples were always within 0% of the certified values.

Samples were then further digested by heating in a microwave oven in four sequential steps (5 min each at 80, 160, 240 and 320 W). After digestion, solutions were diluted by adding 40-ml milli-Q water and metal concentrations were then measured with ICP-MS (UltraMass 700, Varian, Australia). Spectral interference resulting from the dissolved tissues were corrected by means of internal standard method using yttrium for the lighter masses (Cr, Mn, Fe, Co, Ni, Cu, Zn, As) and indium for heavier masses (Cd, Pb) (Taylor et al., 1997; Mubiana et al., 2005). After analysis, metal body concentrations of individual mussels were calculated and expressed on dry tissue weight basis (i.e. μg/g dry weight).

2.3. Modeling the effect of body size

The effect of body size on metal concentration was modelled using power function according to previous studies (Boyden, 1974; Riget et al., 1996; Wang and Fisher, 1997), where tissue metal concentration (Y) is a power function of mussel tissue weight (W) [Eq. (1)].

$$Y = aW^b. \quad (1)$$

The relationship is often transformed using a double log transformation to yield a linear function [Eq. (2)],

$$\log_{10}Y = \log_{10}a + b\log_{10}W \quad (2)$$

where b is the slope of the linear function and $\log_{10}a$ is the Y-intercept. A value of $b > 0$ or <0 shows metal concentration that increases or decreases with animal body size, respectively. If metal concentration is independent of animal body size b is not significantly different from zero.

3. Results

3.1. Effect of body size on metal concentrations

As shown in Fig. 2A, average wet soft tissue weight did not vary significantly between groups (Kruskal–Wallis ANOVA test, $p > 0.05$), except for the lowest group that was heavier than the other groups and there was also a significant difference between the top most group and the group second from the low water mark (0.6 m).

For the determination of the relationship between body tissue weight (g) and metal concentration (μg/g dry weight), pooled data was used in order to increase the sample size. Results of all 10 metals showed decreasing concentration with increase in body weight according to the power function [Eq. (1)] and in order to apply linear regression analysis, the data were plotted on double log transformations [Eq. (2)] and Fig. 3 shows the plots for Pb as example (plots of other metals not shown).

Further analysis was performed to examine the influence of tidal exposure on the regression slope relating metal concentration with body weight. Two groups (i.e. the lowest and the highest placed mussels) were compared (F-test, in GraphPad Prism v4.01) for significance differences in the slopes of the two curves shown in Fig. 4. Results (Table 1), found no differences between the slopes (b) for As, Co, Cr, Cu, Fe and Ni. Therefore, for these metals, pooled slopes based on pooled mussel samples, were computed and ranged from −0.30 (As, Cu) to −0.58 (Ni). In the case of Cd and Pb, higher slopes were recorded in the high tidal mussel group while for Mn and Zn the low tidal mussel group had higher slopes.

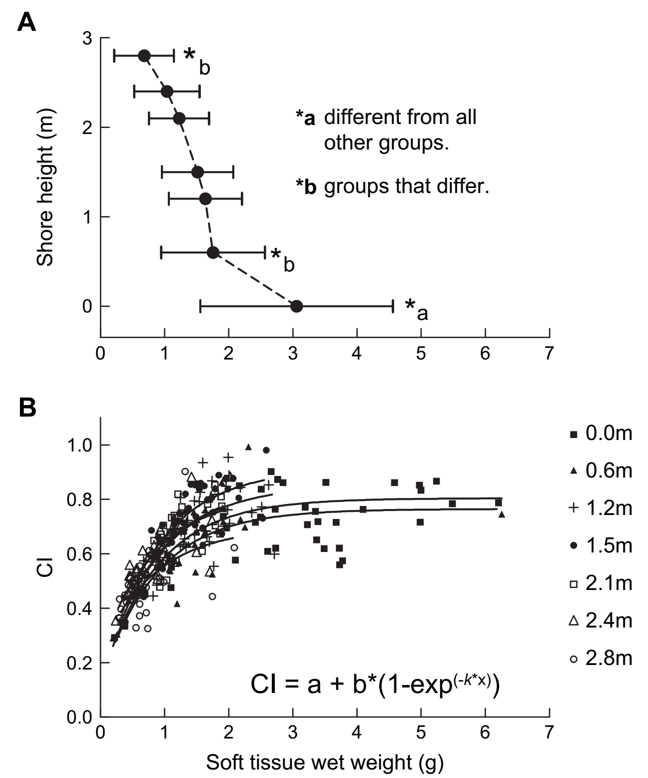

Fig. 2. (A) Mean (±SD) soft tissue wet weight in sample groups from seven different shore heights and (B) condition index of mussels in the seven mussel groups plotted against soft tissue wet weight (g). Data in each group were fitted using non-linear function shown in the figure.

3.2. Effect of condition index (CI) on metal concentrations

Condition index of each individual was determined as a ratio of tissue wet weight (g) to internal cavity volume (cm^3) according to Lobel et al. (1991). In all the groups, CI increased as a function of tissue wet weight towards a plateau (Fig. 2b) and the resulting curves were tested for significant differences using F-test ($p < 0.05$) with null hypothesis '*one curve for all data sets*'. Results showed no differences among individual curves ($F_{18,252}$=1.239; $p = 0.2305$). Therefore, in further analysis where metal concentration was related to CI, pooled data were used after excluding individuals below 1.5 g wet weight, i.e. only the range where CI did not vary with body weight. A linear model was applied in which metal concentration in the tissues was plotted as a linear function of CI (Table 2). Results showed most metals (i.e. Cd, Co, Cr, Cu, Ni, Pb and Zn) were negatively related to CI, explaining between 4% (Cu) and 13% (Ni) of the variations. In the case of As, Fe and Mn there were no significant linear regressions with CI ($p > 0.05$).

3.3. Effect of tidal exposure on metal concentration

To analyse the influence of tidal exposure, metal concentrations in each group were normalized to an average mussel (0.1 g tissue dry weight), using group specific regressions equations. The resulting values were then plotted against shore height as shown in Fig. 5. Spearman's rank correlation

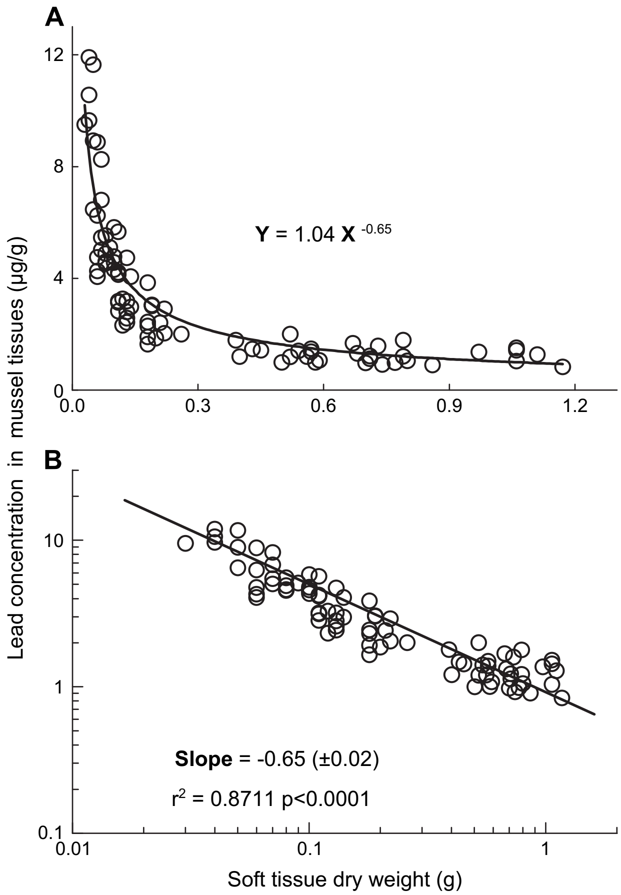

Fig. 3. (A) Relationship between tissue Pb concentration (μg/g dry weight) and soft tissue dry weight (g) and (B) a resulting linear plot from double logarithmic transformation of (A) according to Eq. (2) (see text for details). The value in brackets represents a standard error of the slope and fitting parameters (r^2 and p-levels) are also indicated.

analysis was performed to evaluate the relationship between shore height and tissue metal concentrations. Three patterns were observed, (i) As, Cd and Zn increased with shore height, (ii) Cu, Fe and Mn decreased with shore height and (iii) Co, Cr, Ni and Pb were independent of shore height.

3.4. Influence of body size, CI and shore height in a less heterogeneous sample

Mussels used in biomonitoring studies are usually of similar body size (based on shell length) (Lobel et al., 1991). Therefore, we analysed the significance of the contribution of the three factors towards explaining the variability in the metal concentrations in less heterogeneous samples, i.e. individuals between 1.5 and 2.5 g (wet weight) collected within 1 m shore height. A multiple linear regression model [Eq. (3)] was therefore applied:

$$\text{Metal Conc}_{\text{mussel}} = a + b(\text{TWW}) + c(\text{CI}) + d(\text{TH}) \quad (3)$$

where Metal $\text{Conc}_{\text{mussel}}$ is metal concentration in the soft tissues (μg/g), TWW is tissue wet weight (g), CI is condition index (g/cm^3), SH is shore height (m), b, c and d are coefficients which indicate the contribution of each factor while a is a parameter which explains variability that remains. Results (Table 3) showed that contribution from tissue weight was insignificant for all metals, CI still showed significant contribution for Cd, Co, Cr, Ni, Pb and Zn while shore height was only significant for three metals As, Cd and Zn. Other metals Cu, Fe and Mn showed no significant contribution from all three factors.

4. Discussion

4.1. Influence of body size on metal concentration

For all metals and tidal exposed groups, except Zn in the most exposed, tissue concentrations decreased with increase in body size and in all these cases, the data was best fitted using power function [Eq. (1)] where metal concentration decreased as a power of tissue weight. According to the values of b, Pb in the most exposed group had the strongest effect from body weight and Zn had the least or no effect from body weight in the same group. Generally, these results are consistent with literature concerning *Mytilus edulis* (Boyden, 1974; Riget et al., 1996; Wang and Fisher, 1997), in the related bivalve *Crassostrea gigas* (Mouneyrac et al., 1998) and in marine gastropods (Cubadda et al., 2001). Generally, three patterns have been reported in literature on molluscs, metal concentration that is positive, negative or independent of body weight. However, in *M. edulis* only two types of relationships seem to be prevalent according to available literature (i.e. Boyden, 1974; Lobel and Wright, 1982; Riget et al., 1996; Wang and Fisher, 1997). The most common is tissue concentrations that decrease with increase in body weight and in other cases tissue concentrations are independent of body size.

It is commonly thought that decreasing metal concentration in bigger individuals is caused partly by dilution effect due to unequal growth rate compared to metal accumulation (Newman, 1995). Furthermore, size-specific uptake rate for Cd, Co and Zn and also size-specific Cd elimination rates have been demonstrated in laboratory experiments (Wang and Fisher, 1997). Size-specific metabolism has also been suggested (Boyden, 1974). Another explanation put forward relates to size-specific internal regulation for essential metals (Cubadda et al., 2001). However, results from literature and the current study, have shown no strong evidence to delineate between essential and non-essential heavy metals. Though several metals (As, Co, Cr, Cu, Fe and Ni) showed a consistent slope (b) (i.e. no effect of tidal exposure), other metals like Cd, Mn, Pb and Zn, did not. The later is consistent with a study by Riget et al. (1996) in which size-dependence of metal concentrations varied with location and year.

Results presented here, show that size-effect can be minimised or removed when mussels within a narrow range of body size are sampled. However, the size-dependence for some metals (Cd, Mn, Pb, Zn), can also be influenced by tidal exposure, therefore in monitoring situations, the effects of size should not be accounted for by use of a common regression but a sample-specific regression should be applied.

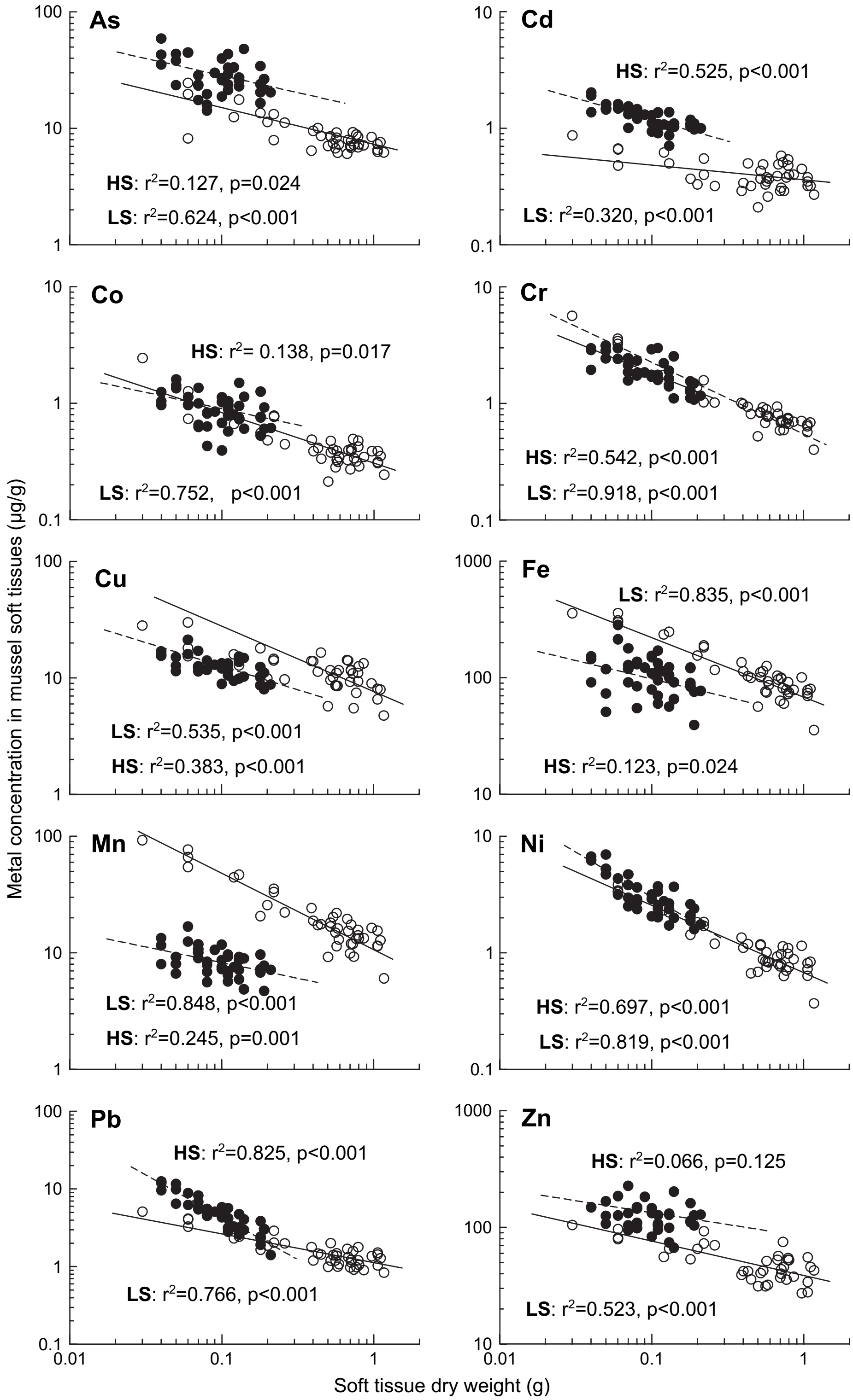

Fig. 4. Metal concentrations (µg/g dry weight) as a function of tissue dry weight (g) in mussel sample from low shore (open circles) and high shore (closed circles). The data were fitted to linear regressions (HS – high shore and LS – low shore).

4.2. Effect of condition index (CI) on metal concentrations

In a heterogeneous sample CI accounted for 4–13% of the metal variability, these values may appear modest, probably because animals used in the study did differ so widely in regard to condition index. Furthermore, in a less heterogeneous sub-sample consisting of individuals of similar tissue weight and shore height, CI still contributed significantly to the remaining variability for Cd, Co, Cr, Ni, Pb and Zn (Table 3).

Table 1
Comparing two regression curves relating metal concentrations and body weight in two mussel samples collected from the lower and upper shores. The data is plotted on double log plots (see text for details). The regression coefficients were compared using the F-test at $p = 0.05$ level. 'NS' denotes a slope not significant different from zero

	Regression slopes		F-value	df	p	Pooled slope
	Low shore	High shore				
As	−0.31	−0.27	0.173	1.78	0.678	−0.30
Cd	−0.18	−0.35	4.153	1.79	0.040	–
Co	−0.44	−0.27	2.82	1.79	0.097	−0.41
Cr	−0.58	−0.48	2.51	1.79	0.118	−0.54
Cu	−0.31	−0.28	0.136	1.80	0.714	−0.30
Fe	−0.52	−0.39	3.32	1.79	0.074	−0.48
Mn	−0.61	−0.30	12.12	1.79	0.001	–
Ni	−0.55	−0.69	2.83	1.78	0.097	−0.58
Pb	−0.42	−1.00	50.58	1.80	<0.001	–
Zn	−0.27	NS	–	–	–	–

The results show the importance of CI towards explaining natural variability observed among individuals of similar body size within the same habitat. Generally, physiological condition is considered one of the main factors with potential to control internal distribution and retention of contaminants in mussels (Widdows and Donkin, 1992). The processes responsible for metal uptake and accumulation in mussels are actively controlled by physiological and biochemical processes. Therefore, any changes in these processes due to a variety of factors such as tidal exposure, body size, sex, reproductive status or nutritional status (Lobel et al., 1991; Smaal and Widdows, 1994), may contribute to variability among individuals.

4.3. The effect of tidal height

Among the many natural factors affecting sessile intertidal organisms, shore height is probably the most important and obvious factor with greatest potential to cause high individual variability in the contents of heavy metals in individuals within a mussel bed. However, the influence of tidal exposure has not received the attention it deserves and in *M. edulis* only few studies involving Cd, Cu, Pb and Zn could be found (Nielsen, 1974; Phillips, 1976; Lobel and Wright, 1982). This is despite the fact

Table 2
Results of a linear regression relating tissue metal concentrations to CI in a mussel sample covering a range between 1.5 and 2.5 g soft tissue wet weight

	Slope	r^2	n	p
As	–	0.013	163	0.143
Cd	−0.912	0.127	162	<0.001
Co	−0.288	0.066	163	0.001
Cr	−0.557	0.104	163	<0.001
Cu	−4.702	0.038	163	0.012
Fe	–	0.017	162	0.098
Mn	–	0.019	163	0.083
Ni	−0.835	0.134	163	<0.001
Pb	−1.562	0.118	163	<0.001
Zn	−54.11	0.055	163	<0.003

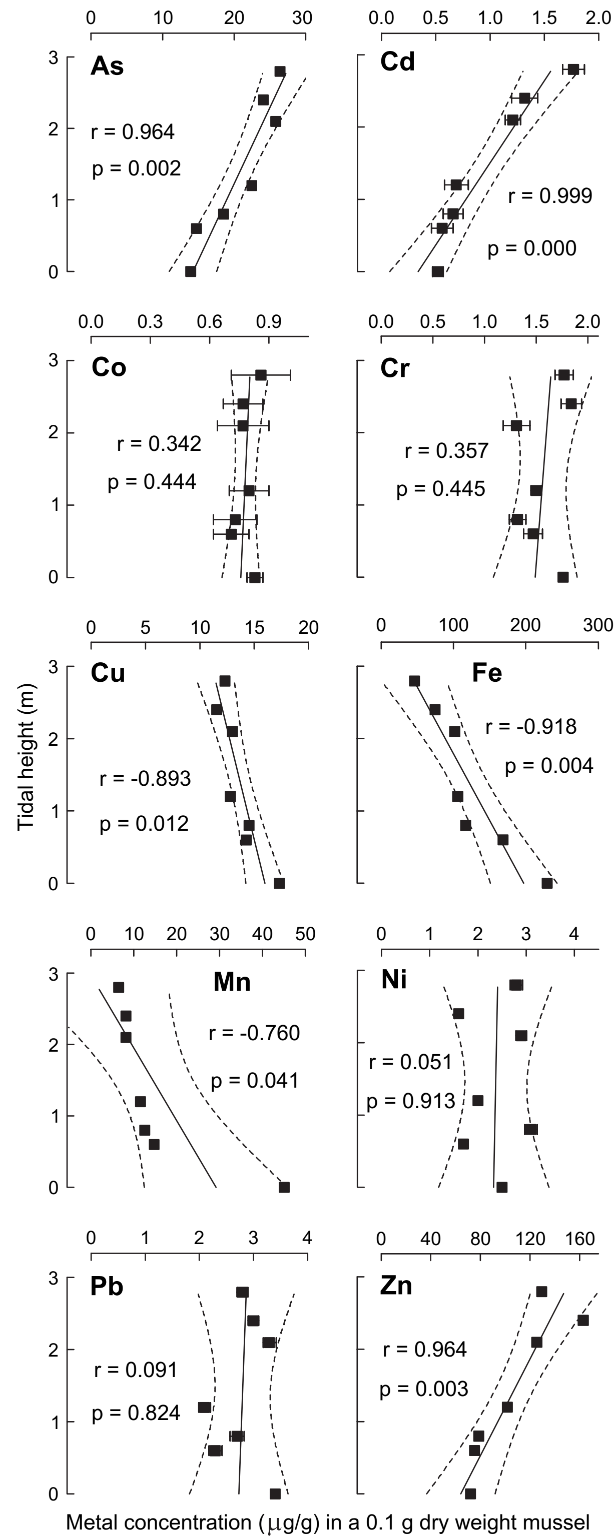

Fig. 5. Metal concentration (µg/g dry tissue weight) normalized to an average mussel of 0.1 g soft dry weight. Mean values and associated standard errors were plotted (x-axis) against shore height (m). Note: for some metals standard errors are too small to be clearly visible.

that prolonged exposure to air exposes organisms to weather elements with obvious consequences on organism's physiology, while periodic submergence can influence bioavailabity of waterborne metals. Impacts on different biological processes like

Table 3
Results of a multiple regression analysis estimating relative contribution to metal variability from tissue weight, CI and shore height represented by parameters *b*, *c* and *d*, respectively. The model also included an additional term *a* for the variability not explained by the three factors

	a	*b*	*c*	*d*	Variation explained (%)
As	8.675	2.575^{ns}	−15.67^{ns}	9.816	59.2
Cd	0.849	0.151^{ns}	−1.170	0.293	60.7
Co	0.576	0.010^{ns}	−0.315	0.091^{ns}	35.2
Cr	1.415	−0.049^{ns}	−0.619	0.057^{ns}	25.0
Cu	16.02	−0.816^{ns}	−3.791^{ns}	−0.188^{ns}	5.0
Fe	93.24	13.79^{ns}	−54.55^{ns}	3.879^{ns}	4.3
Mn	17.08	1.090^{ns}	−7.113^{ns}	−2.535^{ns}	23.6
Ni	1.918	−0.185^{ns}	−0.729	0.048^{ns}	31.7
Pb	2.973	−0.158^{ns}	−1.613	0.099^{ns}	19.3
Zn	101.8	−5.819^{ns}	−45.12	11.64	19.2

Model: $Conc_{mussel} = a + b(\text{tissue weight}) + c(\text{CI}) + d(\text{shore height})$

metabolism, osmoregulation and filtration, all have the potential to influence accumulation and tissue concentrations of metals in mussels (Widdows and Donkin, 1992).

Generally, the influence of tidal exposure on accumulated metals in mussel tissues showed differences that were dependent on the type of metal. Efforts to explain those differences based on whether the metal is essential or non-essential, proved inconclusive. The only indication of a pattern based on essentiality of metals, was the apparent decrease in concentrations in more exposed mussels for the essential metals, Cu, Fe and Mn. However, it is not clear whether it was because of internal regulation of the metals by mussels or was due to decrease in exposure time as mussels were progressively less submerged in water.

Besides biological explanations for the influence of shore height, a presence of a salt wedge in some estuaries may cause differences in metal content of overlaying waters (Phillips, 1976). However, this kind of vertical stratification is not likely in well-mixed waters such as the Scheldt Estuary or the North-Sea. Generally, factors responsible for causing vertical variations in metal concentrations in intertidal mussels are still not well understood.

In view of these results and other studies (i.e. Lobel et al., 1991), it is recommended that for biomonitoring purposes, samples of similar body size be collected close to the water mark during low tide and whenever possible, any other environmental conditions with potential to influence physiological condition should be standardized. However, in situations where these conditions could not be fulfilled, normalization of tissue metal content should be considered to a common body size, condition index or tidal height. Furthermore, since in mussels the relationship between body size and metal concentration can be influenced by external factors (i.e. tidal height − Cd, Mn, Pb and Zn), therefore, normalization of metal concentration to a standard body size should be performed using sample-specific correction factors or regressions.

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