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Microcontaminant accumulation, physiological condition and bilateral asymmetry in zebra mussels (*Dreissena polymorpha*) from clean and contaminated surface waters

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Abstract

Chemical and biological monitoring of pollution in the aquatic environment is essential to assess the quality of surface waters. Zebra mussels (*Dreissena polymorpha*) have been used extensively to monitor pollution in freshwater environments, especially in bioaccumulation studies, whereby pollutant levels in tissues have been used as a measure of exposure. However, there is a need for good biomarkers that reflect the impact of exposure to pollutants. Bilateral asymmetry, commonly used as a measure of developmental instability, has a high potential as a biomarker to monitor stress caused by pollution. Nevertheless, until recently, no studies have evaluated bilateral asymmetry as a biomarker in zebra mussels. Biomarkers related to the energy metabolism may give a good indication of the physiological cost of exposure to pollution.

In this study, we investigated whether the physiological condition (energy reserves and condition indices) and bilateral asymmetry of shells of zebra mussels are potentially useful biomarkers to monitor the impact of micropollution, such as trace metals, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and di(*p*-chlorophenyl) dichloroethylene (*p*,*p*′-DDE) in the freshwater environment. Bilateral asymmetry of the zebra mussel shells was examined with respect to levels of pollutants accumulated in the mussels and compared to the physiological condition of the mussels.

Levels of PCBs and several trace metals (especially Cd, Cu and Zn) were very high in four of the six sampling locations and in some locations the physiological condition of the mussels was significantly depressed. Nevertheless we did not find any relation (on individual or population level) with bilateral asymmetry of zebra mussel shells. Therefore our results suggest that bilateral asymmetry of zebra mussel shells is not a good measure for the impact of pollution in freshwater ecosystems. The energy reserves and condition indices, on the other hand, gave a valuable indication of the physiological condition of zebra mussels and are useful to monitor the impact of pollution if physiological and environmental factors are taken into account.

Keywords: Bilateral asymmetry; Trace metals; Organic micropollutants; Physiological condition; Energy reserves; *Dreissena polymorpha*

1. Introduction

Biomonitoring programs, in which accumulation of pollutants and biomarker responses are measured in organisms, provide the opportunity to determine simultaneously the presence and the impact of pollutants in the environment. Pollutant uptake and accumulation in organisms is dependent on the bioavailability in the environment and therefore, tissue concentrations in some species can be used as a measure of exposure (e.g. fish: Hendriks et al., 1998; midge larvae: Bervoets and Blust, 1999; mussels: Voets et al., 2004).

The impact of accumulated pollutants can be estimated by measuring biomarker responses. Biomarkers are valuable to measure the impact of pollutants if they reflect the health status

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of the organism. Biomarker responses should increase/decrease with increasing toxicity of the exposure and should be related to fundamental life history characteristics, such as growth and reproduction.

Biomarkers related to the energy metabolism are very interesting to reveal toxic stress. Pollutants exert their action at the molecular and cellular level and these perturbations will directly or indirectly influence the energy metabolism of an organism (Marr et al., 1995; Rosen, 2002). Several authors have demonstrated negative effects of pollutants on energy uptake (Kraak et al., 1994a,b; Knops et al., 2001). However, also maintenance cost to compensate for cellular damage, caused by the toxicants, might be increased (e.g. repairing mechanisms, new protein/enzyme synthesis, increasing osmoregulating activities). Also energy invested in detoxification (Van Campenhout et al., 2004; Bebianno et al., 2003) and/or elimination of toxicants (Rosen, 2002) will contribute to an increased maintenance cost. A decreased energy uptake and an increased maintenance cost (for survival) lead to a negative impact on the energy budget, and can result in reduced growth and/or reproduction (De Coen and Janssen, 2003). When more energy is used (e.g. for maintenance, reproduction) than is taken up, the energy reserves will be depleted. Several studies have demonstrated a negative effect of pollutants on the energy budget of organisms (Smolders et al., 2002; Widdows et al., 2002; De Coen and Janssen, 2003). Energy reserves can be quantified by measuring sugar (or glycogen), protein and lipid content.

Besides measuring energy stores, allometric condition indices and physiological stress indices can be used as a biomarker. In particular, the tissue condition index (dry tissue weight/shell weight ratio) and the hydration index (wet tissue weight/dry tissue weight ratio) have been considered as suitable criteria for mussel condition (Mersch and Pihan, 1993; Soto et al., 2000; Smolders et al., 2004).

Based on theoretical and empirical considerations, bilateral asymmetry, the asymmetry between the right and the left sides of bilateral traits, has a high potential as a biomonitoring tool in conservation biology (Leary and Allendorf, 1989; Clarke, 1995; Lens et al., 2001). Bilateral asymmetry is commonly used as a measure for developmental instability (DI), the (in)ability of organisms to buffer their development against small, random perturbations of cellular processes ('developmental noise' Palmer, 1994). The effects of these local perturbations will accumulate on the left and right sides of developing individuals separately, and may give rise to deviations from symmetry in otherwise bilaterally symmetrical characters (Palmer and Strobeck, 1986; Wilson and Manning, 1996). To suppress or buffer the disruptive effect of developmental noise, organisms have evolved homeostatic mechanisms ('developmental stability' Palmer, 1994).

Environmental and genetic stresses have shown to increase bilateral asymmetry in several organisms (Parsons, 1992; Lens et al., 2001). Bilateral asymmetry is easy to measure (right minus left trait values), a wide variety of organisms show bilateral symmetrical traits and there are indications that bilateral asymmetry can be used to measure the impact of stress before populations become irreversibly affected (Lens et al., 2001; Clarke and McKenzie, 1992; Clarke, 1995). Therefore bilateral asymmetry has increasingly been promoted as a general biomarker in conservation biology (Leary and Allendorf, 1989; Clarke, 1995).

In the freshwater environment, an organism particularly interesting to monitor environmental pollution is the zebra mussel (*Dreissena polymorpha*). This bivalve, common in many surface waters, has been used frequently for biomonitoring studies (Hendriks et al., 1998; Smolders et al., 2002; Bervoets et al., 2005). Zebra mussels are efficient accumulators of micropollutants (Hendriks et al., 1998; Bervoets et al., 2005), easy to collect in large numbers and are sedentary, reflecting site specific pollution. They are resistant to a broad range of environmental conditions (Claudi and Mackie, 1993) and to various types of pollution (Bervoets et al., 2005). Zebra mussels are increasingly important in the ecology of surface waters, since they are an important food source for some fish and water birds (Tucker et al., 1996; Zimmermann et al., 1997).

Bivalves are recognized as very useful biomonitoring organisms, but so far, no or very few studies have evaluated effects of pollution on bilateral asymmetry in bivalves. To our knowledge, there are no studies concerning bilateral asymmetry in zebra mussels.

In this study, we investigated whether (i) the physiological condition and (ii) bilateral asymmetry of shells of zebra mussels are potentially useful as indicators of micropollution in the freshwater environment. The physiological condition (energy reserves and condition indices) and bilateral asymmetry of four traits of zebra mussel shells was determined in six populations of zebra mussels including relatively clean locations, locations strongly contaminated with organic pollutants and locations strongly contaminated with trace metals. Bilateral asymmetry of the zebra mussel shells was examined with respect to levels of pollutants accumulated in the mussels and with respect to the physiological condition.

2. Methods

2.1. Study area and sample collection

Zebra mussels were collected in August 2003 in six sites (in Flanders, Belgium) characterized by different types and degrees of micropollution. The selection of the sites was based on tissue levels of contaminants in zebra mussel and eel measured in previous studies (Bervoets et al., 2005). Site M1 in canal Beverlo (Leopoldsburg) and M2 in canal Herentals-Bocholt (Lommel) are severely polluted with heavy metals. Site O1 and O2 in, respectively, the ponds Weerde (Zemst) and Zennegat (Walem) are severely contaminated with organic micropollutants and site C1 and C2 in the ponds Walenhoek (Niel) and Nekker (Mechelen) were expected to be relatively clean and were used as reference sites. In each location, 70 zebra mussels with a shell length between 17 and 29 mm were carefully removed from the substrate and transported in plastic containers with 60 l of water from the sampling site. The pH, oxygen concentration, conductivity and temperature were measured in the sampling locations with a WTW multiline F/SET-3 field kit. Water samples were taken for metal analysis.

From the 70 mussels collected at each location, 45 mussels were divided in three equal groups. Tissues of the mussels from each group were pooled, homogenized (Ultra-Turrax) and sub-samples were taken to determine micropollutant concentrations (metals and organic micropollutants) and biomarker responses, i.e. energy stores and water content. The shells of the mussels were used to determine bilateral asymmetry. The other 25 mussels were treated individually. In these mussels, bilateral asymmetry on shell traits, condition indices and metal concentrations were measured in the tissues of the same mussels. Unfortunately, mussels do not contain enough tissue to measure organic micropollutants on individual level.

2.2. Metal analysis in mussel tissues and water samples

Wet tissues were rinsed with artificial freshwater (294 mg/l $CaCl_2{\cdot}2H_2O$, 123.25 mg/l $MgSO_4{\cdot}7H_2O$, 64.75 mg/l $NaHCO_3$, 5.75 mg/l KCl), blotted dry and weighed. The tissues were dried for 24 h at 60 °C, cooled to room temperature in a desiccator and dry weight was measured to the nearest 0.01 mg on a Mettler AT261 balance. Tissues were digested in a 5:1 mixture of HNO_3 and H_2O_2 according to the microwave digestion procedure as described by Blust et al. (1988). Metal concentrations (Cd, Cu, Cr, Ni, Pb and Zn) were determined by inductive coupled plasma atomic emission spectrometry (ICP-AES, Varian Liberty Series II) and expressed in μg per gram dry weight (μg/g dw). The metals were selected based on metal levels measured in a previous study (Bervoets et al., 2005). Analytical accuracy was determined using process blanks and certified reference material of the Community Bureau of Reference standard for trace elements in mussel tissue (CRM 278). Recoveries ranged between 90% and 106% of the certified values (R.S.D. < 10%). Water samples of the six sampling locations were filtered (0.45 μm), acidified to 3% HNO_3 and the metal concentrations (Cd, Cu, Cr, Ni, Pb and Zn) were analysed by inductive coupled plasma mass spectrometry (ICP-MS, Varian).

2.3. Analysis of organic pollutants

The PCB congeners 28, 31, 52, 74, 95, 99, 101, 105, 110, 118, 128, 132, 138, 149, 153, 156, 163, 170, 177, 180, 183, 187, 194, 196, 199, the PBDE congeners 28, 47, 99, 100, 153, 154, 183, HCB and *p,p′*-DDE, were measured to assess the overall contamination with organic micropollutants. Methods used for the determination of organic pollutants are described briefly below (Bervoets et al., 2005). Mussel tissue was ground with Na_2SO_4, internal standards (BB103, BB155, PCB46 and PCB143) were added and the analytes were extracted with hexane:acetone (3:1, v/v) with a hot Soxhlet manifold. The crude extract was concentrated and subjected to clean-up on acidified silica. Analytes were eluted with *n*-hexane and dichloromethane. The eluate was concentrated and transferred to an injection vial.

PBDEs were determined on an Agilent (Palo Alto, CA, USA) gas chromatograph coupled with a mass spectrometer (GC/MS) operated in electron-capture negative ionisation (ECNI) and equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 capillary column (SGE, Zulte, Belgium). PCBs, *p,p′*-DDE and HCB were determined on an Agilent GC equipped with a micro electron capture detector (ECD) and with a 50 m × 0.22 mm × 0.25 μm HT-8 capillary column. The quality control was done by daily check of calibration curves, regular analyses of procedural blanks, blind duplicate sample analyses, reference material CRM 349 (PCBs in cod liver oil), and internal standards. Recoveries for individual PBDE congeners were between 87% and 104% (R.S.D. ≤ 12%), while recoveries of PCBs, HCB and *p,p′*-DDE ranged between 75% and 90% (R.S.D. < 10%).

2.4. Bilateral asymmetry

2.4.1. Asymmetry measurements

The mussel shells were cleaned thoroughly. They were soaked in 1 M NaOH for 12 h, then carefully scrubbed with a soft toothbrush with 1 M NaOH, rinsed with de-ionized water and dried for 24 h at 60 °C. Bilateral asymmetry was determined on four traits of the left and right shells of the mussels. The length and height were determined by digital photography (Olympus C5050; 30 cm, f 1/8, 1/25 s) and an image processing system (Optimas 6.1). The width was measured with a caliper (0.01 mm) and the weight of the shells was measured on a Mettler AT261 balance to the nearest 0.01 mg.

The received data were ln-transformed to approach normality for subsequent analyses (Graham et al., 2003) and corrected for FA-size associations (Palmer and Strobeck, 2003). This implies that subsequent notations of asymmetry calculations like right minus left sides (R − L) are actually ln(R) − ln(L).

2.4.2. Asymmetry analysis

Measurement error was assessed using two independent repeated measurements of both sides of 20 mussels. For the length and height human measurement error and error due to the photographing process (Pither and Taylor, 2000) were evaluated by photographing both sides of 20 mussels two times, and by measuring each picture two times. Measurement error was quantified by calculating the repeatabilities of asymmetry values as intra-class correlations from the inter-individual variance component in an ANOVA with individual as random effect (Bland and Altman, 1996). For the measurements with the image analysis system, we applied a random effect ANOVA with photographing session nested within individual and the repeated measurements as residual error (Pither and Taylor, 2000). For each trait, the presence of other types of asymmetry was assumed when the signed asymmetries (R − L) showed a significant departure from zero (directional asymmetry) or a negative kurtosis-value (anti-symmetry). When directional asymmetry (DA) was present, this directional component was subtracted from all asymmetry values to obtain FA values reflecting DI (Palmer, 1994). The independence between the size and FA of traits was appraised by a linear regression of the unsigned asymmetries (|R − L|) against individual trait size ((R + L)/2). Finally, developmental independence of the traits under study was evaluated by testing for correlations in signed FA between these traits (Van Dongen et al., 1999), and organism-wide FA was tested with between-trait correlations in unsigned FA (Lens and Van Dongen, 1999).

The individual FA estimates corrected for DA were subsequently used to test for differences in FA between populations and to test for relations between FA and condition indices (TCI and water content) and metal enrichment units (m-EU, see further). This was done for each trait separately after Bonferonni correction for the number of traits tested (Rice, 1989), and for all traits simultaneously while correcting for dependency between traits within individuals in a mixed model (i.e. a composite FA index, Bjorksten et al., 2000).

2.5. Analysis of energy reserves

To determine the energy stores, mussel tissues were homogenized on ice (Ultra-Turrax) in four volumes of de-ionized water (MQ). The obtained homogenates were split in sub-samples for the analyses of the protein, sugar and lipid content. Protein content was determined with the Bradford assay (1976). First, proteins were precipitated with 15% trichloroacetic acid (TCA). After centrifugation (10 min, 1000 × *g*, 4 °C), the supernatant was transferred to another vial and kept for the sugar measurements. The pellet was washed with 5% TCA and after centrifugation (10 min, 1000 × *g*, 4 °C) the supernatant was added to the previous supernatant fraction. Subsequently, the pellet was dissolved in NaOH, incubated for 30 min at 60 °C and neutralized with HCl. The samples were diluted and 25 μl of sample was transferred in triplicate to a multiwell plate. Two hundred and fifty microlitres of Bradford solution (BIORAD diluted five times) was added and after 5 min the absorption was measured at 592 nm. Bovine serum albumin (BSA) (Sigma, USA) was used as a standard.

The sugar content was measured on the supernatant fraction according to De Coen and Janssen (2003). The supernatant was incubated with phenol and H_2SO_4 for 30 min. Samples were transferred to a multiwell plate and the absorption was measured with a Spectra microplate reader (492 nm). Glucose was used as standard.

Lipid content was determined according to Bligh and Dyer (1959). Briefly, the homogenates were vortexed with chloroform, methanol and purified water. After centrifugation (5 min, 10,000 rpm, 4 °C), the top phase and the membrane were discarded, and 100 μl of the chloroform phase was incubated with H_2SO_4 for 15 min at 200 °C. The absorption was measured at 340 nm. Tripalmitin (Sigma, USA) was used as a standard.

2.6. Condition indices

The tissue condition index (TCI) was calculated by dividing the dry weight of the tissue by the volume of the mussel. The mussel volume (Vol_m) was calculated based on the length, width and height of the mussel with the formula:

$$Vol_m = \frac{\text{length} \times \text{width} \times \text{height}}{C}$$

The constant *C* was determined empirically as follows. The volume of 134 zebra mussels with a length between 17 and 29 mm was measured with a graduated cylinder to the nearest 0.25 ml. The measured volume (Vol_m) was plotted against the volume of the cube obtained by multiplying the length × width × height (Vol_{Cube}). The regression line $Vol_{Cube} = C \times Vol_m$ was calculated and the slope (constant *C*) amounts to 2.77 ± 0.03 ($N = 134$, $R^2 = 0.909$, $p < 0.001$).

The water content of the mussels was determined by the ratio of the wet weight/dry weight.

2.7. Statistics

Analyses of variance (ANOVA, with post hoc Duncan's multiple range test), Kruskal–Wallis test and correlation matrices were used to analyze the data on micropollutants, energy stores and condition indices, as appropriate. If necessary, data were tested for homogeneity of variance by the log-ANOVA test and for normality by the Kolmogorov–Smirnov test for goodness of fit. The tests were performed with STATISTICA 5.0 (StatSoft Inc.). Differences were considered significant when *p*-values were <0.05. Statistical methods used are outlined in Sokal and Rohlf (1998). The data of bilateral asymmetry were analysed as described above (asymmetry analyses).

3. Results

3.1. Physico-chemical characteristics

The physical and chemical conditions of the water in the sampling locations are presented in Table 1. The pH values were comparable with levels previously measured in these locations (Bervoets et al., 2005). Trace metal concentrations in the water were low, except for Cd in location M1. The Pb concentrations in the water were below the detection limit in all the locations.

Table 1
pH, temperature (*T*), conductivity (cond), oxygen concentration (O_2) and heavy metal concentrations (Cd, Cu, Ni, Pb and Zn) in the water of the six sampling sites in Flanders (Belgium)

Location	Code	pH	Temperature (°C)	Cond (μs/cm)	O_2 (mg/l)	Cd (μg/l)	Cu (μg/l)	Ni (μg/l)	Pb (μg/l)	Zn (μg/l)
Niel	C1	8.2	21.2	910	8.8	0.0	0.8	2.9	DL	2.0
Nekker	C2	8.1	25.1	554	9.1	0.2	0.6	1.5	DL	0.2
Weerde	O1	8.2	23.4	524	8.4	0.1	0.8	2.6	DL	1.2
Zennegat	O2	8.6	27.0	734	8.7	0.1	2.2	3.0	DL	3.8
Beverlo	M1	8.2	21.8	696	9.4	1.3	16.4	4.5	DL	103.2
Lommel	M2	7.8	21.7	774	7.8	0.5	4.7	4.3	DL	33.7

DL, value under detection limit.

Table 2
Heavy metal concentrations in the tissues of *Dreissena polymorpha*, collected at the six sampling sites in Flanders (Belgium)

Location code	Mean ± S.D. (μg/g dw)				
	Cd	Cu	Ni	Pb	Zn
C1	1.17 ± 0.31 a	6.00 ± 1.47 a	2.18 ± 1.25 a	1.08 ± 0.99 a	73.7 ± 14.5 a
C2	6.51 ± 1.22 b	10.68 ± 2.88 b	8.01 ± 3.23 b	1.08 ± 0.82 a	88.7 ± 19.2 b
O1	1.27 ± 0.30 a	11.70 ± 3.20 b	3.58 ± 1.93 a	3.54 ± 2.20 a	89.3 ± 13.3 b
O2	2.16 ± 0.53 a	13.55 ± 3.78 b	6.25 ± 3.58 b	2.07 ± 1.73 a	95.8 ± 24.0 b
M1	23.55 ± 6.58 d	28.47 ± 7.50 d	8.19 ± 3.86 b	15.17 ± 7.75 c	185.1 ± 34.1 d
M2	14.93 ± 4.01 c	23.38 ± 7.57 c	10.64 ± 5.22 c	6.65 ± 4.11 b	143.4 ± 19.1 c

Values represent the mean ± S.D. of 25 mussels. Metal concentrations are expressed as μg/g dry weight (μg/g dw). Metal concentrations in the mussels from the different locations differ significantly unless they have the same letter (ANOVA, Duncan's post hoc, $N = 25$).

3.2. Metal accumulation

Metal concentrations in the zebra mussels ranged from 0.68 to 34.82 μg/g for Cd, from 3.26 to 49.68 μg/g for Cu, from 0.43 to 17.81 μg/g for Ni, from <0.2 to 30.35 μg/g for Pb and from 45.61 to 242.28 μg/g for Zn. Metal concentrations measured in the pooled and individual mussel samples in the different location separately were very similar and no significant differences were found for any of the metals (Wilcoxon matched pairs test). As expected, the heavy metal concentrations in the mussels differed strongly between the sampling locations. The metal concentrations (mean ± S.D.) measured in the individual mussels are presented in Table 2. Mussels from C1, O1 and O2 contained the lowest metal concentrations. Mussels from M1 and M2 accumulated high concentrations of Cd, Cu, Ni, Pb and Zn. Unexpectedly, mussels from C2, selected as a reference location, had also elevated Cd and Ni concentrations.

The enrichment of the metals in the zebra mussels was calculated with metal enrichment units (m-EU-values). The m-EU-values in zebra mussel tissue are calculated by dividing the individual metal concentrations in the tissues by background metal concentrations in zebra mussels from the Ysselmeer in The Netherlands (values in Ysselmeer in ng/g ww: Cd 63, Cu 1100, Pb 86, Zn 15,000). The Ysselmeer is considered a clean location in terms of chemical pollution. For some metals, there were no values reported in the zebra mussels from the Ysselmeer. For those metals the concentrations in zebra mussels from Niel were used as background concentration. Finally the retrieved values for the different metals were summed. The m-EU-values in zebra mussels at the six sampling locations are shown in Fig. 1a. m-EU-values were highest in mussels from M1 and M2. Mussels from C1, O1 and O2 had very low m-EU-values and no significant differences were observed between these locations. Mussels from C2 had significantly elevated m-EU-values compared to mussels C1, O1 and O2.

3.3. Accumulation of organic micropollutants

The concentrations of the organic pollutants in zebra mussels from the six locations are presented in Table 3. The PCB congeners that were present in the mussels in the highest concentrations were PCB138, PCB149 and PCB153. Concentrations of almost all the PCB congeners in the mussels were correlated. Concentrations of PCB congeners with similar octanol–water partition coefficients (K_{ow}) were strongly correlated. Correlations between the congeners with lower numbers (from PCB28 to PCB170) and between the congeners with the higher numbers (from PCB177 to PCB199) had correlation coefficients of at least 0.7 ($N = 6$).

Also the PBDEs in the mussels were correlated and the sum of the PBDEs was correlated with the sum of the PCBs

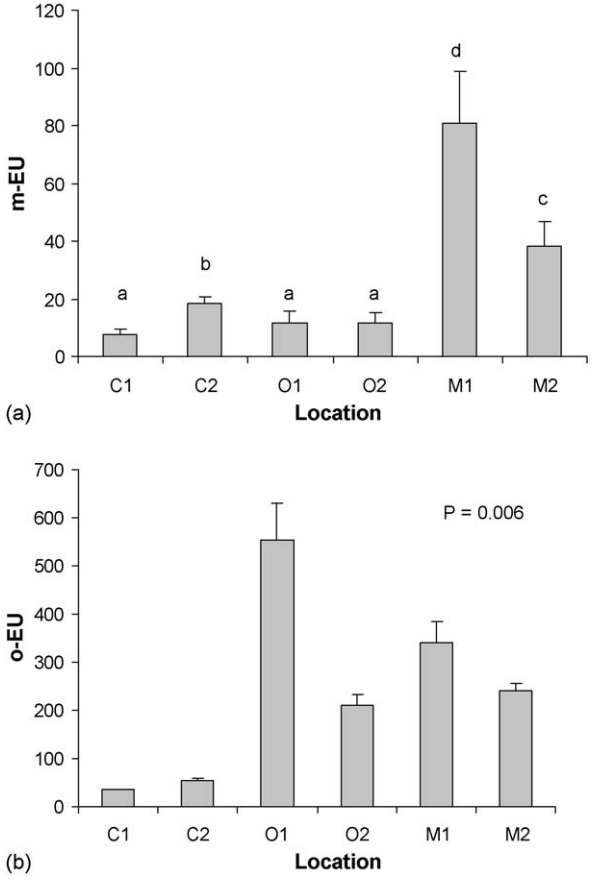

Fig. 1. (a) Metal enrichment units (m-EU; mean ± S.D.) in zebra mussels from the six sampling locations in Flanders (Belgium). The enrichment units in the mussels from the different locations are significantly different unless they have the same letter (ANOVA, Duncan's post hoc, $N = 25$); (b) enrichment units for organic pollutants (o-EU; mean ± S.D.) in zebra mussels from the six sampling locations in Flanders (Belgium). The enrichment units in the mussels differ significantly between different locations (Kruskal–Wallis, $H(5, N = 18) = 16.25$, $p = 0.006$).

Table 3
Concentrations of organic pollutants (PCBs, PBDEs, HCB and *p,p′*-DDE) in *Dreissena polymorpha* from six sampling sites in Flanders (Belgium)

PCB number	DL	Mean ± S.D. (ng/g ww)					
		C1	C2	O1	O2	M1	M2
PCB28	0.15	DL	DL	6.16 ± 0.72	1.32 ± 0.06	1.60 ± 0.18	1.08 ± 0.15
PCB31	0.15	DL	DL	3.48 ± 0.65	1.08 ± 0.20	0.76 ± 0.09	0.32 ± 0.08
PCB52	0.20	0.25 ± 0.05	0.51 ± 0.09	8.88 ± 1.08	3.89 ± 0.28	3.29 ± 0.30	2.43 ± 0.39
PCB74	0.15	DL	0.1 7 ± 0.03	3.00 ± 0.59	1.75 ± 0.19	0.65 ± 0.09	0.46 ± 0.05
PCB95	0.15	0.54 ± 0.14	0.71 ± 0.23	9.25 ± 1.97	3.60 ± 0.48	4.71 ± 0.63	4.32 ± 0.14
PCB99	0.15	0.21 ± 0.02	0.41 ± 0.07	6.30 ± 0.97	2.23 ± 0.47	2.23 ± 0.34	1.77 ± 0.13
PCB101	0.15	0.35 ± 0.11	0.87 ± 0.16	16.54 ± 2.36	5.01 ± 0.99	6.44 ± 0.89	4.82 ± 0.50
PCB105	0.15	DL	0.22 ± 0.06	3.37 ± 0.83	1.87 ± 0.32	0.66 ± 0.05	0.26 ± 0.06
PCB110	0.20	0.26 ± 0.01	0.91 ± 0.18	13.02 ± 2.09	4.66 ± 0.76	5.50 ± 0.70	3.51 ± 0.17
PCB118	0.20	0.42 ± 0.02	0.72 ± 0.17	10.68 ± 1.96	3.95 ± 0.60	2.91 ± 0.34	2.11 ± 0.17
PCB128	0.10	0.12 ± 0.01	0.35 ± 0.08	5.90 ± 0.62	2.21 ± 0.23	3.63 ± 0.46	1.90 ± 0.06
PCB132	0.15	DL	0.46 ± 0.09	7.18 ± 1.09	2.44 ± 0.22	4.91 ± 0.59	3.57 ± 0.23
PCB138	0.15	0.58 ± 0.07	1.25 ± 0.24	17.62 ± 1.92	5.59 ± 0.69	8.00 ± 1.09	5.69 ± 0.32
PCB149	0.15	0.25 ± 0.01	1.02 ± 0.22	14.70 ± 2.23	4.90 ± 0.66	10.78 ± 1.45	8.10 ± 0.32
PCB153	0.15	0.85 ± 0.05	1.56 ± 0.30	22.44 ± 2.63	7.39 ± 0.98	14.04 ± 1.83	11.78 ± 0.78
PCB156	0.15	DL	DL	1.53 ± 0.28	0.57 ± 0.08	0.59 ± 0.08	0.30 ± 0.11
PCB163	0.15	0.21 ± 0.02	0.46 ± 0.07	5.70 ± 0.80	1.99 ± 0.38	3.44 ± 0.47	2.58 ± 0.23
PCB170	0.10	0.11 ± 0.01	0.21 ± 0.04	3.71 ± 0.40	1.63 ± 0.21	2.27 ± 0.26	1.14 ± 0.04
PCB177	0.10	DL	DL	2.19 ± 0.42	0.82 ± 0.08	2.05 ± 0.30	1.43 ± 0.06
PCB180	0.10	0.20 ± 0.02	0.39 ± 0.07	7.51 ± 0.88	3.39 ± 0.44	6.12 ± 0.76	3.16 ± 0.13
PCB183	0.10	DL	0.18 ± 0.04	2.56 ± 0.17	1.20 ± 0.13	2.82 ± 0.36	2.10 ± 0.22
PCB187	0.10	DL	0.29 ± 0.08	4.85 ± 0.33	1.95 ± 0.23	5.76 ± 0.78	4.22 ± 0.14
PCB194	0.10	DL	DL	0.65 ± 0.12	0.33 ± 0.06	0.76 ± 0.09	0.34 ± 0.04
PCB196	0.10	DL	DL	1.57 ± 0.14	0.72 ± 0.08	2.28 ± 0.31	1.00 ± 0.87
PCB199	0.10	DL	DL	0.89 ± 0.12	0.45 ± 0.06	1.18 ± 0.17	0.65 ± 0.04
Sum PCBs	3.45	4.22 ± 0.39	10.59 ± 2.21	179.7 ± 25.04	64.92 ± 7.71	97.38 ± 12.42	69.02 ± 3.89
BDE28	0.01		0.05 ± 0.01	0.04 ± 0.01	DL	DL	DL
BDE47	0.01	0.05 ± 0.02	0.12 ± 0.03	0.25 ± 0.04	0.12 ± 0.01	0.35 ± 0.06	0.41 ± 0.06
BDE99	0.01	0.03 ± 0.01	0.12 ± 0.02	0.19 ± 0.03	0.13 ± 0.01	0.34 ± 0.06	0.32 ± 0.04
BDE100	0.01	0.02 ± 0.01	0.03 ± 0.00	0.05 ± 0.01	0.03 ± 0.00	0.10 ± 0.01	0.11 ± 0.01
BDE154	0.02	DL	0.02 ± 0.01	0.05 ± 0.01	DL	0.05 ± 0.01	0.04 ± 0.00
BDE153	0.02	DL	0.02 ± 0.01	0.07 ± 0.01	0.03 ± 0.00	0.06 ± 0.01	0.05 ± 0.01
BDE183	0.02	DL	DL	0.03 ± 0.01	DL	0.04 ± 0.01	0.04 ± 0.02
Sum PBDEs	0.09	0.10 ± 0.03	0.34 ± 0.08	0.68 ± 0.10	0.30 ± 0.03	0.94 ± 0.16	0.96 ± 0.13
HCB	0.20	DL	DL	DL	DL	0.36 ± 0.03	0.28 ± 0.03
p,p′-DDE	0.35	0.70 ± 0.05	0.54 ± 0.11	6.49 ± 1.45	2.03 ± 0.41	1.93 ± 0.32	1.42 ± 0.14

The concentrations are expressed in ng/g wet weight (ng/g ww) and presented as the mean ± standard deviation (S.D.) of three replicates of 15 pooled mussels.

($R^2 = 0.348$). PBDE28, PBDE183 and HCB were near the detection limit in mussels from all the locations. Tissue concentrations of PCBs, PBDEs and *p,p′*-DDE differed between mussels from the six locations (Kruskal–Wallis, PCBs: $H(5, N = 18) = 16.25$, $p = 0.0062$; DDEs: $H(5, N = 18) = 16.251$, $p = 0.0069$; PBDEs: $H(5, N = 18) = 15.409$, $p = 0.0088$). Mussels from O1 contained the highest PCB and DDE concentrations, however mussels from O2, M1 and M2 had also strongly elevated PCB concentrations. PBDE concentrations were highest in mussels from M1 and M2. To reduce the amount of variables, the enrichment units for organic micropollutants (o-EU) were calculated by analogy with the method for metals as described above (Fig. 1b) (values in Ysselmeer in ng/g ww: PCB28 0.2, PCB31 0.19, PCB52 0.42, PCB74 0.11, PCB105 1.1, PCB110 0.88, PCB118 0.58, PCB128 0.15, PCB138 1.1, PCB149 1.4, PCB153 2.0, PCB156 0.07, PCB170 0.39, PCB180 0.1, PCB187 0.55, PCB194 0.07, HCB 0.11, DDE 0.54). o-EU-values differed significantly between the locations (Kruskal–Wallis, $H(5, N = 18) = 16.25$, $p = 0.006$). Zebra mussels from location O1 had the highest o-EU-values, followed by mussels from M1, M2 and O2.

3.4. Physiological condition

The physiological condition of the mussels was determined by measuring the energy reserves (sugar, protein and lipid content) and two condition indices (tissue condition index and water content). The mean sugar, protein and lipid concentrations in zebra mussels from the six sampling locations are presented in Fig. 2a–c. The mussels from the different locations showed significant differences in sugar, protein and lipid concentration (Kruskal–Wallis, $p < 0.05$, Fig. 2a–c). Mussels from M2 (strongly polluted with metals, PCBs and DDE) had lower lipid, protein and sugar concentrations than mussels from the other locations. The lipid and sugar concentration was highest in mussels from C1 (reference location). The protein concentration was highest in mussels from M1, however differences in protein

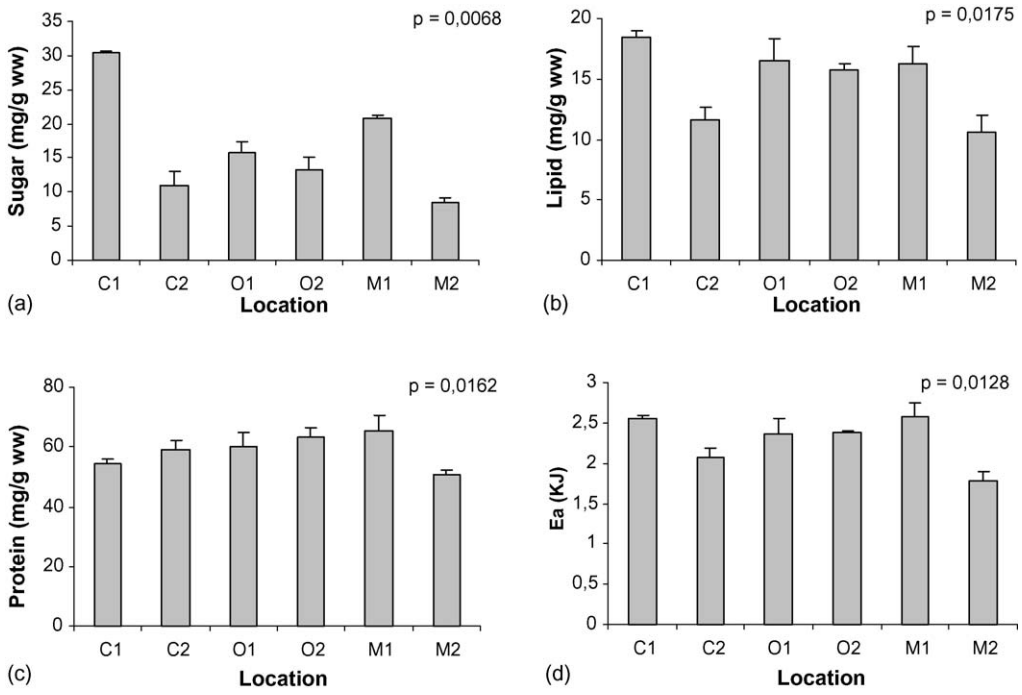

Fig. 2. Sugar (a), lipid (b) and protein (c) concentration and the calculated available energy (E_a) (d) in mussels from six sampling locations in Flanders (Belgium). Each bar shows the mean ± standard deviation of three replicates of 15 pooled mussels. Differences between mussels from the six locations are tested with Kruskal–Wallis, analyses of variance and considered significant if $p < 0.05$.

concentration were rather small in mussels from the different locations. The total energy available in the mussels was calculated by transforming the different energy reserve fractions into energetic equivalents using the enthalpy of combustion (Gnaiger, 1983): 17,500 mJ/mg carbohydrates, 24,000 mJ/mg protein and 39,500 mJ/mg lipid. Mussels from C2 and M2 had less energy available than mussels from the other locations (Fig. 2d).

When the data on energy reserves of all locations were pooled, the sugar and lipid concentration in the mussels was significantly correlated (Table 4). The protein concentration was not significantly correlated to the lipid and sugar concentrations (Table 4).

The TCI and the water content in zebra mussels from the six sampling locations are presented in Fig. 3a and b. Mussels from the six sampling locations showed significant differences in TCI and water content (ANOVA, Duncan's post hoc test, $N = 25$, $p < 0.05$). Mussels from C1 had the highest TCI and mussels from C2 and M2 had the lowest TCI. The water content was lowest in mussels from C1 and highest in mussels from C2 and M2. When the data of the individual measured mussels of all the locations were taken together, the TCI and the water content were significantly correlated ($R^2 = 0.492$, $N = 140$, $p < 0.001$). Correlations between the metals accumulated in the tissues and the TCI and water content are presented in Table 5. The TCI was only significantly correlated with the Ni concentration. The water content was correlated with the Ni and Zn concentration, although the relation with Zn was rather weak.

Correlations between energy stores and condition indices (pooled samples) are presented in a correlation matrix (Table 4). The sugar and lipid concentration are strongly positively correlated to the TCI and strongly negatively related to the water content.

Energy reserves (protein, lipid and sugar concentration), total available energy, TCI and water content were not significantly correlated to the m-EU- and the o-EU-values (correlation, $N = 6$, $p > 0.05$).

Table 4
Correlation matrix shows correlations between the energy stores (sugar, lipid and protein concentrations) and condition indices (TCI and water content) in *Dreissena polymorpha* from six sampling locations in Flanders (Belgium) ($N = 6$)

	Sugar	Lipid	Protein	E_a	TCI
Lipid	0.846^{*}				
Protein	0.063^{NS}	0.380^{NS}			
E_a	0.812^{*}	0.939^{**}	0.611^{NS}		
TCI	0.864^{*}	0.975^{***}	0.393^{NS}	0.943^{**}	
Water content	-0.865^{*}	-0.954^{**}	-0.299^{NS}	-0.895^{*}	-0.985^{***}

Significant correlations are shown with asterisks ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$); ^{NS}non-significant.

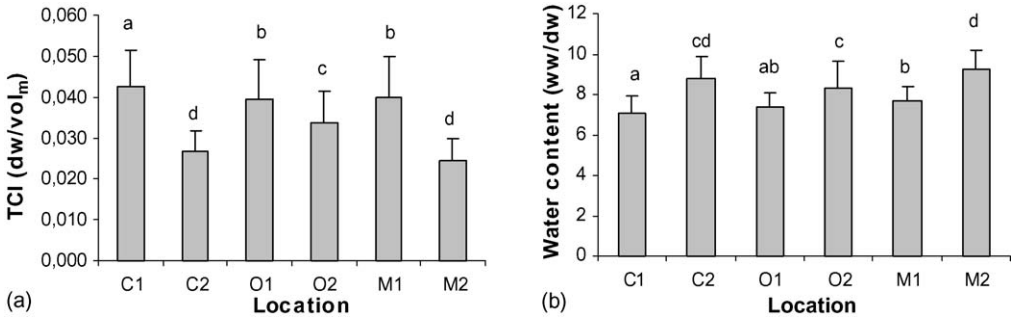

Fig. 3. The tissue condition index (TCI) (a) and the water content (b) of zebra mussels from the six sampling locations in Flanders (Belgium). The bars show the mean ± standard deviation of 25 mussels ($N = 25$). Values are significantly different unless they have the same letter (ANOVA, Duncan's post hoc, $p < 0.05$).

Table 5
Correlation matrix shows correlations between condition indices and the metal concentrations in *Dreissena polymorpha* tissue

	Cd	Cu	Ni	Pb	Zn
Water content	0.110^{NS}	0.189^{NS}	0.663^{***}	-0.035^{NS}	0.203^{NS}
TCI	-0.099^{NS}	−0.142	-0.462^{***}	0.056^{NS}	-0.112^{NS}

Twenty-five zebra mussels are collected in six locations in Flanders (Belgium) ($N = 150$). Significant correlations are shown with asterisks ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$); ^{NS}non-significant.

Table 6
Statistics of the various possible confounding effects of FA as an estimator of DI for the different shell traits of *Dreissena polymorpha*

Trait	Photo	R^2	Measure	Average (R − L)	Student's t	Kurtosis	Size-dependency
Length	99.7		99.9	−0.0007 (0.0006)	-11.0^{***}	1.03	$F_{1,411} = 0.00^{NS}$
Width	99.9		99.9	0.04 (0.002)	23.6^{***}	0.93	$F_{1,411} = 0.52^{NS}$
Height		99.7		−0.05 (0.003)	-14.6^{***}	1.45	$F_{1,440} = 0.14^{NS}$
Weight		99.9		0.0005 (0.002)	0.3^{NS}	1.86	$F_{1,437} = 0.00^{NS}$

The repeatabilities (R^2) of the calculated asymmetries, with the two sources of error for the image analysis measurements partitioned out (see text). The average signed asymmetry (R − L) and the test if this average deviates significantly from zero (Student t-test). The kurtosis value and the F-test for a significant relation between FA and size. Significance levels are shown with following symbols; ^{NS}non-significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3.5. Bilateral trait asymmetry analysis

Repeatabilities of the calculated asymmetries (Table 6) all exceeded 99%, implying that the various potential sources of error were very low. There was no size-dependency for any of the traits under study as expected when applying a log transformation (Table 6), and anti-symmetry appeared to be absent, as kurtosis values of the asymmetries of all traits were positive (Table 6). Directional asymmetry (DA) was present in all traits except the weight measurements (Table 6). The direction of DA differed however between traits, with left side shells being on average more elongated and higher, but narrower than right side shells (Table 6). There were significantly positive correlations in signed FA between shell height, weight and length and a significantly negative correlation in asymmetry between shell width and length (Table 7). These significant correlations in signed FA resulted in a pattern of significant positive correlations between these traits in the unsigned asymmetry, which should not be confused with an individual asymmetry parameter here. The FA-values for the different traits in the six sampling sites are reported in Table 8.

Mussels from the sampling locations did not differ significantly in unsigned FA in any of the traits (all $p > 0.1$ after Bonferonni correction), and neither in the composite FA index ($F_{5,411} = 1.75$; $p = 0.1$), in spite of strong differences in trace metal and organic micropollution levels between the locations. Investigation of the relations between FA and metal load and the two condition indices (water content and TCI), measured on individual mussels, resulted in one significant relation between height FA and water content (Table 9) that remained significant after Bonferonni correction (correcting for 15 tests results in a p-value of 0.01). Zebra mussels with higher water content seem to exhibit less FA (regression slope of -0.27 ± 0.08). This relation did not differ between locations ($F_{5,132} = 0.73$; $p = 0.6$).

Table 7
Correlation matrix of signed and unsigned FA between the different shell traits of *Dreissena polymorpha*

	Width	Length	Height	Weight
Width		-0.11^{*}	-0.06^{NS}	0.01^{NS}
Length	0.15^{**}		0.28^{***}	0.40^{***}
Height	0.02^{NS}	0.15^{**}		0.58^{***}
Weight	-0.06^{NS}	0.26^{***}	0.33^{***}	

The Pearson correlations in signed FA (R − L) are shown in the upper, and the Pearson correlations in unsigned FA (|R − L|) are shown in the lower triangular. Significance levels of the correlations are shown with following symbols; ^{NS}non-significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Table 8
Average unsigned FA-values (FA $|\ln(R) - \ln(L)|) \pm$ S.D., corrected for directional asymmetry, for four traits of mussels shells from mussels collected in clean and polluted surface waters

Location	Ln(FA)			
	Length	Width	Height	Weight
C1	-5.08 ± 1.07	-4.22 ± 1.22	-3.21 ± 1.09	-3.62 ± 1.02
C2	-5.02 ± 1.46	-3.77 ± 0.90	-3.44 ± 1.11	-4.09 ± 1.25
O1	-4.99 ± 0.98	-4.16 ± 1.00	-3.28 ± 1.10	-4.12 ± 1.06
O2	-4.93 ± 0.91	-4.09 ± 1.03	-3.65 ± 1.23	-3.94 ± 0.98
M1	-4.91 ± 1.08	-3.90 ± 0.99	-3.42 ± 1.19	-3.71 ± 1.07
M2	-5.28 ± 1.17	-4.11 ± 1.36	-3.64 ± 1.07	-4.04 ± 1.08

Table 9
Relation between the different shell traits of *Dreissena polymorpha* and individual condition indices (water content and TCI) and metal load (m-EU)

Trait	m-EU	Water content	TCI
Length	$F_{1,123} = 0.36^{NS}$	$F_{1,123} = 0.08^{NS}$	$F_{1,126} = 0.38^{NS}$
Height	$F_{1,123} = 1.90^{NS}$	$F_{1,123} = 0.52^{NS}$	$F_{1,126} = 0.93^{NS}$
Width	$F_{1,133} = 0.45^{NS}$	$F_{1,142} = 11.4^{***}$	$F_{1,135} = 2.51^{NS}$
Weight	$F_{1,133} = 2.76^{NS}$	$F_{1,142} = 1.53^{NS}$	$F_{1,135} = 0.27^{NS}$
Composite index	$F_{1,123} = 1.84^{NS}$	$F_{1,132} = 3.84^{NS}$	$F_{1,127} = 0.20^{NS}$

Significance levels of the regression slopes are shown with following symbols; ^{NS}non-significant; $^{***}p < 0.001$.

4. Discussion

Zebra mussels are tolerant to a wide range of environmental conditions, giving them the opportunity to invade many freshwater systems in Asia, Europe and North-America (Claudi and Mackie, 1993). In Flanders (Belgium), zebra mussels are present in most canals, ponds, lakes and drinking water reservoirs. The wide distribution of zebra mussels makes them suitable as a biomonitoring organism for freshwater ecosystems. In this study, polluted and reference sites were selected in ponds and canals. The sampling locations had some differences in physico-chemical characteristics, but overall, conditions of the water were all in the range to allow good survival and growth for zebra mussels. Therefore, these environmental conditions are not likely to be a source of stress to the zebra mussels in this study (Claudi and Mackie, 1993). Heavy metal concentrations in the water were low and except for Canal Beverlo (M1), all below the Flemish water quality standards (1 μg Cd/l, 200 μg Zn/l, 50 μg Cu/l, 50 μg Ni/l; Flemish Government, 2000). Nevertheless, in two sampling locations (M1 and M2), zebra mussels accumulated high amounts of metals compared to values reported in literature (Camusso et al., 1994; Cope et al., 1999; Bervoets et al., 2005). Trace metals easily bind to suspended matter and sediments and as a consequence, water concentrations are often low, though metal bioavailability from the aquatic ecosystem can be significant (Roper et al., 1996; Redeker and Blust, 2004).

Organic micropollutants like PCBs, PBDEs, DDE and HCB are lipophilic and have high K_{ow} values and therefore concentrations of organic pollutants in the water were too low to measure in this study. Nevertheless tissue concentrations of most PCBs, PBDEs and DDE were detectable in mussels from all the locations using approximately 1–2 g of tissue. Only HCB and some PBDE congeners were below the detection limit in most locations. The PCB concentrations in mussels from Weerde (O1), Zennegat (O2), Beverlo (M1) and Lommel (M2) were very high compared to concentrations in mussels from the reference locations and concentrations reported in literature (Bervoets et al., 2005; Hendriks et al., 1998; Cope et al., 1999). Levels of sumPCB and DDE were, respectively, 30 and 10 times higher in mussels from the most polluted location Weerde than in the mussels from the reference location Niel. PBDE concentrations were highest in mussels from Beverlo (M1), Lommel (M2) and Weerde (O1) being approximately 7–10 times higher compared to mussels from Niel. The PBDE concentrations in the zebra mussels measured in this study are comparable with the PBDE concentrations measured in zebra mussels from other water bodies in Flanders and The Netherlands (Bervoets et al., 2005; de Boer et al., 2003). The enrichment values for organic micropollutants (o-EU-values) indicate that mussels from Weerde (O1) were most polluted, followed by mussels from Beverlo (M1), Lommel (M2) and Zennegat (O2). Mussels from Niel (C1) and Nekker (C2) had only slightly elevated o-EU-values.

Based on pollutant levels in the zebra mussels, we can conclude that locations Beverlo (M1) and Lommel (M2), selected as metal polluted locations, were not only severely polluted with trace metals, but also with organic micropollutants. Location Weerde (O1) and Zennegat (O2) were strongly polluted with organic micropollutants, but were not polluted with trace metals. The reference location Niel can be considered as a good reference location, since accumulated concentrations of metals and organic pollutants were low compared to literature. The location Nekker was also relatively clean except for some Cd and Ni in the mussel tissue.

To determine the physiological condition of zebra mussels we measured the energy stores and two condition indices. The lipid content and the body mass have shown to be directly related to reproductive output and survival and can therefore be considered as valuable markers to evaluate the 'health' or 'condition' of zebra mussels (Sprung, 1995; Stoeckmann and Garton, 2001). The tissue condition index (TCI) is an index that uses the dry weight of mussels normalized for the size of the mussels. This dry weight/shell weight ratio has been used as an estimation of the physiological condition in mussels (Pampanin et al., 2005; Smolders et al., 2004); however we found significant differences in shell weight/shell volume ratios in zebra mussels from the different locations, indicating that the thickness of the shells varied between the different locations. Therefore, we opted for shell volume, calculated as the product of length, width and height of the mussels divided by 2.77 (see Section 2), to normalize the dry weight for size of the mussels. A high TCI is an indication of good condition. On the other hand, high water content is an indication of bad condition and/or osmotic stress. (Carini et al., 1995, 1999).

In this study, mussels from the reference location Niel had the highest sugar content, lipid content, TCI and had the lowest water content and therefore we can conclude that these mussels had the best condition. On the other hand, mussels from Lommel, which contained very high concentrations of trace metals and organic

micropollutants, had strongly decreased sugar (decreased with 72.4 ± 2.8%), lipid (decreased with 42.2 ± 7.2%) and protein content (decreased with 6.7 ± 2.9%) and, as a result, a strongly decreased level of available energy in their tissues (decreased with 30.4 ± 4.1%) compared to mussels from the reference location Niel. They also had the lowest TCI and the highest water content. All these biomarkers indicate that mussels from Lommel (M2) had a very poor condition. Mussels from Weerde (O1) and Zennegat (O2), which contained mainly organic pollutants in their tissues (PCBs, PBDEs and DDE), also had slightly decreased condition. Levels of sugar and lipids were decreased with, respectively, 56.3 ± 5.9% and 14.4 ± 2.8% in mussels from Zennegat (O2) and with 48.4 ± 5.6% and 9.9 ± 4.4% in mussels from Weerde compared to the reference location Niel. Although the organic micropollution status in Weerde (O1) is very high compared to other water systems, the zebra mussels seem to suffer only moderately from pollution with PCBs, PBDEs and DDE. Toxicity data of PCBs and PBDEs in general and especially under field conditions are scarce. In zebra mussels Roper et al. (1996) found no effect of sediments highly contaminated with mainly polycyclic aromatic hydrocarbons (PAHs) and PCBs on zebra mussel survival, while *Daphnia magma*, exposed to the same sediments showed highly significant increases in mortality. In marine mussels, some effects of organic micropollutants on enzyme activities and scope for growth were observed (Shaw et al., 2002), however effects seemed to be rather attributed to other micropollutants than PCBs, like aliphatic and/or aromatic hydrocarbons (Sole et al., 2000; Widdows et al., 2002).

Unexpectedly, mussels from Beverlo (M1) had a better physiological condition than mussels from Lommel (M2), although they accumulated more micropollutants (trace metals, PCBs and DDE) in their tissues. Nevertheless compared to the reference location Niel, these mussels had a decreased sugar, lipid and TCI and a higher water content, reflecting decreased physiological condition. The relatively small impact of trace metals on the physiological condition in mussels from Beverlo might be explained by a higher metal detoxification capacity in mussels from Beverlo by induction of metal binding proteins. Metallothionein like proteins (MTLP) levels were up to 3.2 times higher in mussels from Beverlo than in mussels from Lommel (unpublished results), which might be an indication of acclimatization or adaptation. Mussels from Nekker, which were only contaminated with Cd and Ni (and PBDE28 was elevated), had according to the biomarker results a surprisingly poor condition. The water level fluctuated substantially in this location, giving them an additional stress caused by a temporary exposure to air.

Levels of bilateral asymmetry of mussels from the six sampling locations were determined on four traits of zebra mussel shells. Correlation in signed FA indicated that weight, height and length were to some degree developmentally integrated. The correlations of height and length with weight are not remarkable as they are presumably confounded by shell size variations as longer and higher shells weigh more. The positive correlation between height and length suggests that these different traits share underlying developmental pathways (Van Dongen et al., 1999; Klingenberg et al., 2001). A significant negative correlation in signed FA between two traits, as found here for length and width, however, has never been documented, and suggests a developmental dependence in an unexpected way. This might be explained in the context of the closing mechanism of the mussel, in which both shell sides interact. Additional evidence for this explanation comes from the observed directional asymmetries in opposite directions for shell width and length. Although being very subtle, the closing of the valves of zebra mussels is asymmetric on the ventral (flattened) side, with a small overlap of the right valve over the left valve, anterior and posterior from the permanent opening through which the byssal apparatus extends. Also near the hinge (elastic ligament), the valves are asymmetric, with the left valve hinging slightly over the right valve, which might be related to the observed directional asymmetry in length and width. The negative correlation in signed FA between length and height might be caused by small deviations in the exact position of the hinge between the individuals and the possibility of zebra mussels to adjust the development of their valves to ensure optimal closure.

No clear differences in FA were found between locations despite the high variation in environmental pollution between sites. There are several reasons why the FA of the shell traits under study did not provide a reliable indication of environmental pollution. First, the mussels might have adapted to the local stress conditions during the evolutionary history of the zebra mussel population (Hoffman and Parsons, 1991). This explanation is not likely, especially not for all locations since the measurements of the physiological condition of the mussels show clear indications of stress in mussels from some polluted locations (e.g. Lommel) compared to mussels from the reference location. Second, there could be a differential mortality whereby the lower quality mussels, with higher levels of FA, fail to reach sizes large enough to sample (Lens et al., 2002). Zebra mussels also occur in suboptimal conditions, where growth and reproductive success are lower and mortality rates are higher. Mortality of low quality individuals might decrease developmental instability on population level if the stressor itself is not causing an increase in fluctuating asymmetry. This was observed by Polak et al. (2002), who found that *Drosophila melanogaster* reared at high concentrations of As, at which flies suffered significant mortality, showed reduced fluctuating asymmetry. Positional FA was positively correlated with the amount of flies emerging per bottle. FA of flies reared at sub-lethal dosage did not differ from that in the controls. They conclude that FA-based monitoring, especially of potentially lethal forms of stress, can lead to erroneous conclusions about the health and well being of a population. Although differential mortality can decrease FA on population level, in this study, we also found no relation on the individual level. FA values of the shells were not related to the metal concentrations measured in the same individuals, indicating that differential mortality cannot fully explain the observed results.

Third, as mentioned above, both shell sides of a mussel may not grow independently, as they both make up the closing of the mussel. If so, developmental instability is probably masked as higher levels of FA are compensated during development to

keep the closure relatively watertight. This would be in support with the compensatory growth hypothesis (Emlen et al., 1993) or the residual asymmetry hypothesis formulated by Kellner and Alford (2003), which states that there are compensatory mechanisms that act to counter the effects of developmental noise. According to Kellner and Alford (2003) there are two ways that these mechanisms might operate: (i) negative feedback among cells might act to suppress biosynthesis on the site that is too large and (ii) positive feedback between left and right structures (Emlen et al., 1993) via the nervous or circulatory systems might maintain symmetry by promoting catch-up growth on the lagging site (Emlen et al., 1993).

Finally, Badyaev et al. (2005) states that functionally integrated traits have a weaker response to stress because asymmetry on these traits might enable the individuals functioning.

Bilateral asymmetry has been evaluated as a measure of stress caused by overpopulation and food depression in marine mussel and oyster farms (Fréchette and Daigle, 2002; Fréchette et al., 2003) and they also found no significant effect of these stresses on bilateral asymmetry. Kiflawi et al. (2000) studied the relation between FA and the geographical range of mollusk populations. They found some indications that mollusks in peripheral populations exhibit more developmental instability than mollusks in central populations of their geographical range.

When we related individual FA of the different traits with individual measures of condition and metal load, only a weak negative relation between height FA and water content was found. Increased water content is an indication of bad condition and therefore, this negative correlation between FA and water content is unexpected. This rather contradictory result is also observed in two other studies concerning bivalves. Fréchette and Daigle (2002) observed in Iceland scallops that increasing asymmetry values for anterior and posterior pairs of ears were associated to lower mortality. They found no relation between FA and growth. Also in cultured oysters, Fréchette et al. (2003) found, in a study concerned with the relation between morphological features of the shells and mortality, higher FA values in living than in dead individuals.

5. Conclusion

Levels of organic micropollutants and several trace metals, especially Cd, Cu and Zn, were very high in four of the six sampling locations. The different physiological biomarkers gave consistent results and a good indication of the physiological condition of the mussels. We observed a significant decreased physiological condition in mussels from polluted locations and yet we did not find any effect on fluctuating asymmetry on four traits of zebra mussel shells. It seems that zebra mussels have compensatory mechanisms during development to ensure good closure of the shells.

Our results suggests that FA of the measured traits, height, length, width and weight are not useful to monitor trace metal pollution (Cd, Cu and Zn), PCBs, PBDEs and DDE. However, it is possible that FA is related to specific pollutants, other than measured in this study, or that effects on FA only appear at even higher concentrations. In the latter case, FA of zebra mussel shells is not sensitive enough for routine monitoring of pollution in freshwater ecosystems. The lack of a relation between FA and the energy stores of the mussels, which is a very important marker with respect to survival and reproduction, indicates that asymmetric growth of the shells is not a good measure to determine the "general health" of the zebra mussels. The energy reserves and condition indices on the other hand gave a valuable indication of the physiological condition of zebra mussels and are valuable to monitor the impact of pollution if physiological and environmental factors are taken into account.

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