

P. S. Rainbow · W. Fialkowski · A. Sokolowski
B. D. Smith · M. Wolowicz

Geographical and seasonal variation of trace metal bioavailabilities in the Gulf of Gdansk, Baltic Sea using mussels (*Mytilus trossulus*) and barnacles (*Balanus improvisus*) as biomonitors

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Abstract The barnacle *Balanus improvisus* and the mussel *Mytilus trossulus* have been used as biomonitors of the trace metals Cu, Zn, Cd, Fe, Pb, Mn and Ni at five sublittoral sites in the Gulf of Gdansk (Baltic Sea) between February 2000 and September 2001. The study has established a benchmark against which future biomonitoring programmes will be able to establish changes in local metal pollution, particularly if metal loadings in the river Vistula (draining into the Gulf) alter in the future. The study highlighted differences in trace metal bioavailabilities to both barnacles and mussels, geographically and over time. Accumulated metal concentrations of Cu, Zn, Fe, Pb and Ni, but not Cd or Mn, were correlated in the barnacles and mussels, suggesting that the bioavailabilities of the former metals to the two biomonitors were similar. The barnacles showed greater discriminatory power than the mussels as trace metal biomonitors. Concentrations of trace metals in surficial sediments (<63 µm) did not correlate significantly with accumulated metal concentrations in either barnacles or mussels, indicating that sediment metal concentrations are not necessarily good proxy measures of ambient trace metal bioavailabilities to the local coastal filter feeders.

Introduction

Trace metals have the potential to cause ecotoxicological effects in coastal habitats if present in sufficiently high availabilities, usually as a result of anthropogenic activities (Phillips and Rainbow 1994; Szefer 2002). The Gulf of Gdansk, at the southern end of the Baltic Sea, is a coastal habitat at risk from anthropogenic toxic metal contamination (Szefer 2002). The Gulf is partly enclosed, being bordered to the north by the Hel peninsula and to the west and south by continental Poland (Fig. 1). The three adjacent cities of Gdynia, Sopot and Gdansk emit effluent into the Gulf, as does the River Vistula, which drains most of Poland including the major industrial regions and cities of Katowice, Krakow and Warsaw. The Vistula is a key source of trace metals (e.g. cadmium, copper, lead, silver and zinc) into the Gulf of Gdansk, with further cadmium and lead being introduced by atmospheric transport, and cobalt, iron, manganese and nickel by natural erosion processes (Szefer et al. 1995, 1996; Renner et al. 1998; Szefer 2002). Not surprisingly, the Gulf is considered to be metal-polluted (Szefer 1990, 2002; Glasby and Szefer 1998; Szefer et al. 1998).

This study has set out to investigate the geographical variation of the bioavailabilities of the trace metals copper, zinc, cadmium, iron, lead, manganese and nickel in the Gulf of Gdansk, and how those bioavailabilities vary with season, specifically between February 2000 and September 2001. Bioavailabilities may be modelled by geochemical extraction procedures but can only be measured using biomonitors, for biomonitors provide integrated measures of the bioavailable metal in the habitat, in short, the ecotoxicologically significant fraction of ambient metal in that habitat (Phillips and Rainbow 1994; Rainbow 1995). Strictly, the accumulated concentration of a trace metal in a particular biomonitor will be a measure of the bioavailability of the metal, summed across all sources, to that specific

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P. S. Rainbow (✉) · B. D. Smith
Department of Zoology, The Natural History Museum,
Cromwell Road, London, SW7 5BD UK
E-mail: psr@nhm.ac.uk
Tel.: +44-207-9425275
Fax: +44-207-9425054

W. Fialkowski
Department of Hydrobiology, Jagiellonian University,
ul. Gronostajowa 3, 30-387 Krakow, Poland

A. Sokolowski · M. Wolowicz
Laboratory of Estuarine Ecology,
Institute of Oceanography, University of Gdansk,
al. Pilsudskiego 46, 81-378 Gdynia, Poland

Fig. 1 Sites of collection of *Balanus improvisus* and *Mytilus trossulus* in the Gulf of Gdansk, on the Baltic coast of Poland (see Table 2 for details)



biomonitor. It is therefore preferable to use more than one biomonitor in a programme in order to increase the strength of generalised conclusions to be made about different degrees of metal pollution (Phillips and Rainbow 1994; Rainbow 1995).

This study has used two sympatric biomonitors, the mussel *Mytilus trossulus* and the barnacle *Balanus improvisus*. A preliminary study (Rainbow et al. 2000) has confirmed that the use of these two species as trace metal biomonitors can show up geographical and temporal differences in the bioavailabilities of trace metals in the Gulf of Gdansk. This study leads on from that preliminary validation exercise. Mussels and barnacles take up and accumulate trace metals from solution and from food, in both cases from suspended material, living plankton and detritus in the water. They might therefore be expected to reflect similar metal bioavailabilities in the Gulf, although differential selection of particle sizes and differences in digestive physiology will cause differences in metal bioavailabilities to the two species (Wang 2002).

It is intended that this study will lay down a benchmark against which future biomonitoring programmes will be able to trace changes in metal pollution in the Gulf of Gdansk, particularly if future industrial effluents emitted into the Vistula have reduced toxic metal loads. The study will also act as a model for similar investigations of coastal and estuarine regions receiving high loads of anthropogenic trace metal input. Furthermore, the data will allow a comparison of the relative bioavailabilities of different trace metals to mussels and barnacles, and their relative powers of discrimination as biomonitors of trace metals. Finally, the availability of data from sediment samples collected simultaneously has enabled a comparison of the results obtained from each biomonitor with those from the sediment analyses.

Materials and methods

The mussel *M. trossulus* Gould (Mollusca, Bivalvia) and the barnacle *B. improvisus* Darwin (Crustacea, Cirripedia) were collected by dredge from five sublittoral sites in the Gulf of Gdansk, on the coast of Poland (Fig. 1), between February 2000 and September 2001 (Table 1) and stored frozen. Details of the sites are provided in Table 2.

The soft tissues were dissected from 10 mussels from each site on each sampling occasion, rinsed briefly in distilled water and dried at 60°C in individual pre-weighed acid-washed polythene vials. According to availability, the bodies of 5 to (usually) 10 barnacles were also dissected out, rinsed and pooled into each of 5 to (usually) 10 samples from each site in such vials and dried at 60°C. Samples were subsequently dried to constant weight, digested in Aristar concentrated HNO₃

Table 1 *Balanus improvisus* and *Mytilus trossulus*: dates (day of month) of collection of barnacles and mussels from each of five sites on up to 11 sampling occasions between February 2000 and September 2001

	Puck	Mechelinki	Gdynia	GN Buoy	Vistula plume
2000					
February		22		22	
March				31	31
May	09	11	11	11	11
July	12	12	12	25	25
September	11	11	11	13	13
December		6	6	6	
2001					
January		15	15	16	16
March		15		15	15
May	25	25	8	8	8
July	26	30	26	26	30
September	4	4	4	3	3

Table 2 *B. improvisus* and *M. trossulus*: details of sites of collection of barnacles and mussels in the Gulf of Gdansk, February 2000 and September 2001

	Position	Depth (m)	Salinity range	Temperature range (°C)
Puck	54° 40.4' N, 18° 30.5' E	5	6.5–7.7	12–21
Mechelinki	54° 37.1' N, 18° 32.5' E	7	6.7–7.6	2–20
Gdynia	54° 32.8' N, 18° 36.2' E	12	6.9–7.5	2–18
GN Buoy	54° 32.0' N, 18° 48.1' E	38	6.7–7.6	2–15
Vistula plume	54° 25.9' N, 18° 58.8' E	40	6.7–7.7	3–16

(BDH, Poole, UK) at 100°C, and made up to 2 ml with double-distilled water for analysis of concentrations of trace metals (Cu, Zn, Cd, Fe, Pb, Mn, Ni) on an International Laboratory IL-157 atomic absorption spectrophotometer (AAS). Samples of TORT-1 and TORT-2 lobster hepatopancreas certified reference material (National Research Council, Canada) were analysed simultaneously (Table 3), and agreement is considered satisfactory.

As described by Rainbow et al. (2000), invertebrates often show an effect of body size on accumulated trace metal concentrations, and the power function $y = ax^b$ is used here as a suitable model of the relationship between metal concentration (y) and body dry weight (x). Data were therefore transformed logarithmically (to the base 10), converting the power function to a linear relationship for comparison of best-fit regression lines by analysis of covariance (ANCOVA). ANCOVA was employed to test for significant differences in the transformed trace metal concentrations after allowance for differences in the transformed dry weights, with the precondition that there was no significant difference between the regression coefficients (slopes). In the case of the barnacles, the mean body weight of individuals in a pooled sample was used in the analyses.

On sampling occasions between May 2000 and September 2001, samples of the (oxic) surficial sediment were also taken from the dredge using acid-washed

polyethylene tubes. Sediment samples were then dried at 55°C to constant weight and divided into two subsamples. Total organic matter content was computed from the loss in weight of a subsample after combustion at 550°C for 6 h (Kramer et al. 1994). The second subsample was sieved through a 63 µm nylon mesh (Szefer et al. 1995) and the fraction smaller than 63 µm was leached with 1M HCl for 2 h (Suprapur, Merck). This extraction technique allowed determination of the labile and potentially bioavailable metal phases (Bryan and Langston 1992).

All statistical testing was carried out using STATISTICA for Windows (StatSoft 1997). All data used in parametric tests were transformed to logarithms (base 10).

Results

Effect of individual sizes

Regression analyses (not shown) carried out on all log-transformed data sets for each metal confirmed that there was a significant effect ($P < 0.05$) of individual size (dry weight) on metal concentration in at least one data set (either for a separate site or for all sites combined) for each metal for both the barnacle and the mussel data. There was therefore a need to correct for size effects before comparisons of accumulated metal concentrations. ANCOVA was therefore used to compare accumulated metal concentrations intraspecifically between sites and between sampling occasions, by comparing the elevations of best-fit regression lines, having confirmed no significant difference ($P > 0.05$) between the regression coefficients in each comparison.

Because of the presence of a size effect in both the barnacle and mussel data sets, it is meaningless to quote mean metal concentrations for either animal from each site at each sampling date in any comparisons. The relevant concentration that allows comparison is the weight-adjusted mean concentration, which is the metal concentration (as estimated from the best-fit double log regression for each site on each sampling occasion) of the barnacle body or mussel soft tissue of mean weight for the whole data set analysed for that metal. In practice all seven metals were analysed in nearly all samples, although in some cases (particularly for lead and nickel) a few samples may have been

Table 3 Comparisons of mean ($\pm 95\%$ confidence limits, $n = 5$) measured and certified ($\pm 95\%$ tolerance limits) trace metal concentrations ($\mu\text{g g}^{-1}$) in certified reference material [TORT-1 and TORT-2 lobster hepatopancreas (NRC, Canada)]

	Measured	Certified
TORT-2		
Copper	109 \pm 13	106 \pm 10
Zinc	191 \pm 14	180 \pm 6
Cadmium	29.5 \pm 2.5	26.7 \pm 0.6
Iron	117 \pm 11	105 \pm 13
Manganese	12.6 \pm 2.9	13.6 \pm 1.2
Nickel	3.2 \pm 1.2	2.5 \pm 0.2
TORT-1		
Copper	409 \pm 18	439 \pm 22
Zinc	175 \pm 11	177 \pm 10
Iron	191 \pm 14	186 \pm 11
Lead	9.4 \pm 0.6	10.4 \pm 2.0
Manganese	23.9 \pm 1.0	23.4 \pm 1.0
Nickel	2.4 \pm 0.3	2.3 \pm 0.3

Table 4 *B. improvisus* and *M. trossulus*: ranges of weight-adjusted mean metal concentrations ($\mu\text{g g}^{-1}$ dry weight) at each of five sites on up to 11 sampling occasions between February 2000 and September 2001, estimated from regressions of \log_{10} (metal concentration) ($\mu\text{g g}^{-1}$) against \log_{10} (individual dry weight) (g)

	Puck	Mechelinki	Gdynia	GN Buoy	Vistula plume
<i>B. improvisus</i>					
Copper	23.7–39.3	23.6–49.3	31.6–61.7	37.3–63.5	51.5–70.8
Zinc	3,293–14,106	4,466–14,386	6,088–10,048	4,197–7,448	5,610–12,217
Cadmium	5.8–9.3	4.0–12.7	3.7–10.8	4.5–16.5	6.0–14.2
Iron	818–2421	1,474–2,664	1,718–4,405	989–5,395	1,216–3,666
Lead	23.8–43.0	16.0–56.1	13.5–52.4	17.9–76.0	18.0–51.1
Manganese	88.0–399	31.5–237	67.2–217	94.5–186	70.3–238
Nickel	8.0–32.1	6.9–39.2	9.3–34.7	10.7–61.7	8.7–41.4
<i>M. trossulus</i>					
Copper	6.02–8.39	6.96–9.19	7.77–9.36	6.67–10.06	6.94–11.40
Zinc	83.8–130	103–192	98.1–153	61.1–136	96.1–187
Cadmium	2.2–4.3	3.1–5.5	2.5–5.0	2.6–4.7	3.4–5.8
Iron	219–1,027	342–836	366–2,049	476–4,074	586–4,100
Lead	3.8–13.1	5.3–13.4	7.0–15.0	7.4–13.9	6.6–14.0
Manganese	20.3–55.2	13.0–45.8	15.1–97.8	20.7–85.1	25.5–72.7
Nickel	2.0–5.0	2.5–5.7	2.2–5.3	2.6–5.6	2.6–7.8

Table 5 *Balanus improvisus*: statistical comparisons by analysis of covariance (ANCOVA) at $P=0.05$ of accumulated trace metal concentrations in barnacles from up to five sites in the Gulf of Gdansk, Poland on up to 11 sampling dates, as modelled by straight line least squares regressions of \log_{10} (metal concentration) ($\mu\text{g g}^{-1}$) against \log_{10} (individual dry weight) (g). Barnacles from sites showing the same number in a **row** against one date for one

metal do not differ significantly in the concentration of that metal between sites (decreasing order of concentrations is 1 > 2 > 3, etc.). Barnacles collected on dates showing the same letter in a **column** under one site for one metal do not differ significantly in the concentration of that metal between dates (decreasing order of concentrations is A > B > C, etc.)

Metal/Date	Puck	Mechelinki	Gdynia	GN Buoy	Vistula plume
Copper					
February 2000		A	1	A, B, C, D	1
March 2000				A	1
May 2000	B	3	C	3	A, B
July 2000	C	3	D	3	A
September 2000	B, C	4	B	3	A, B, C
December 2000			B	3	D
January 2001			B	2	2, 3
March 2001			B	2	D, E
May 2001	A	2	C	2	A, B
July 2001	A	2	A, B	1, 2	C, D, E
September 2001	A	1, 2	B	2	A, B
Zinc					
February 2000		B, C	1		A, B
March 2000					1
May 2000	C, D	3	C, D	2, 3	A, B
July 2000	D	4	D	3, 4	1, 2
September 2000	C	3	B, C, D	2	B, C
December 2000			A, B	1	2, 3
January 2001			A	1	C
March 2001			A, B	1, 2	4
May 2001	B	1	B, C, D	2	B, C
July 2001	B	1, 2	A, B	2, 3	2
September 2001	A	1	A	1, 2	A, B
Cadmium					
February 2000		A	2		A
March 2000					A, B
May 2000	A	2, 3	A	1, 2	B
July 2000	B, C	1	B, C	1	1
September 2000	C	3	B, C	2	C
December 2000			D, E	1	3
January 2001			E	2	E, F
March 2001			E	2	F
May 2001	C	1, 2	C, D	2	1
July 2001	A, B	2	C	3	D, E
September 2001	A, B	1	A, B	1	C, D, E

Table 5 (Contd.)

Metal/Date	Puck	Mechelinki	Gdynia	GN Buoy	Vistula plume
Iron					
February 2000		A, B, C	1	D, E, F	1
March 2000				C, D, E	1
May 2000	B, C	2	A	1, 2	A
July 2000	C	3	A, B, C, D	2	D, E
September 2000	D	4	A, B	2, 3	B, C, D
December 2000			A	2	A
January 2001		A, B, C	2	D	2
March 2001		B, C, D	1		B, C
May 2001	A	2	D	3	D, E
July 2001	A, B	1, 2	A, B, C	2	A, B
September 2001	C	3	C, D	3	E
Lead					
February 2000		A	2		A
March 2000					B, C
May 2000	A	1, 2	A, B	1, 2	A
July 2000	B	2	C	1, 2	B, C
September 2000	B	2, 3	B, C	1	C, D
December 2000			D, E	2	E, F
January 2001			F	2	F, G
March 2001		E, F	2		G
May 2001	B	1, 2	D	3	F, G
July 2001	A	2	C	3	D, E
September 2001	A	1	A, B	2, 3	D, E
Manganese					
February 2000		B	1		B, C
March 2000					A
May 2000	B, C	1	B	2	A, B
July 2000	C	1	B, C	1, 2	A
September 2000	C, D	1, 2	C, D	1, 2	A, B
December 2000			D	1	A
January 2001			D	1	A, B
March 2001			D	1, 2	B
May 2001	D	2	E	2	A, B
July 2001	A, B	1	A, B	2	A, B
September 2001	A	1	A	2	A, B
Nickel					
February 2000		A	1		A, B, C, D
March 2000					B, C, D
May 2000	A, B	1	A, B, C	1	C, D
July 2000	C	2	B, C	2	A, B, C
September 2000	C	2	A	1, 2	D
December 2000			A	1, 2	A
January 2001		A, B	1	A, B	A, B, C
March 2001		A, B	2		A, B
May 2001	A	1	A, B	2	A, B, C
July 2001	B, C	1	C	1, 2	A, B, C
September 2001	B, C	1, 2, 3	A, B	3	C, D

beneath detection limits. The mean dry weights of either barnacle bodies or mussel soft tissues that were therefore used in calculations were nearly (but not absolutely) the same for each metal. Thus the mean individual dry body weight of the barnacles analysed was 1.32 mg for Cu, Zn, Cd, Fe and Mn, and 1.33 mg for Pb and Ni. In the case of the mussel, the individual soft tissue dry weight corresponding to the weight-adjusted mean metal concentration was 188 mg for Cu, Cd, Fe, Pb and Mn, 189 mg for Ni and 190 mg for Zn.

Tables 4, 5 and 6 and Figs. 2 and 3 summarise the relevant data for *B. improvisus* and *M. trossulus*. The figures show the weight-adjusted mean metal concentrations of the barnacles (Fig. 2) and mussels (Fig. 3)

at each site on each sampling occasion. Table 4 gives the ranges of these concentrations to allow comparisons against data collected in other studies. Tables 5 (barnacles) and 6 (mussels) summarise the results of ANCOVA comparisons between sites on each sampling occasion (geographical differences), and between different samples collected from the same site on different sampling occasions (temporal differences).

B. improvisus: geographical variation

For each of the seven trace metals there is evidence of differences in accumulated metal concentrations

Table 6 *M. trossulus*: statistical comparisons by ANCOVA at $P=0.05$ of accumulated trace metal concentrations in mussels from up to five sites in the Gulf of Gdansk, Poland on up to 11 sampling dates, as modelled by straight line least squares regressions of $\log_{10}(\text{metal concentration}) (\mu\text{g g}^{-1})$ against $\log_{10}(\text{individual dry weight}) (\text{g})$. Mussels from sites showing the same number in a **row**

against one date for one metal do not differ significantly in concentration of that metal between sites (decreasing order of concentrations is $1 > 2 > 3$, etc.). Mussels collected on dates showing the same letter in a **column** under one site for one metal do not differ significantly in concentration of that metal between dates (decreasing order of concentrations is $A > B > C$, etc.)

Metal/Date	Puck		Mechelinki		Gdynia		GN Buoy		Vistula plume	
Copper										
February 2000			A, B	2			B, C	1		
March 2000							A, B, C	1	A	1
May 2000	A, B	3	A	1, 2, 3	A	2, 3	B, C	1, 2	A, B	1
July 2000	A, B	2	A, B	2	A	2	A	1	A	1
September 2000	B	3	A	2	A	2	A	1	A	1
December 2000			A, B	2	A	1, 2	B, C	1		
January 2001				3	A	2, 3	A, B	1, 2	A	1
March 2001			A, B	2			A, B, C	1	A	1
May 2001	A	1, 2	A, B	2	A	1, 2	B, C	1	A, B	1
July 2001	A	1, 2	B	2	A	1, 2	C	1	B	1
September 2001	A, B	2	A, B	2	A	2	A, B	1	A, B	1
Zinc										
February 2000			A, B	1			A, B	1		
March 2000							A, B	1	A	1
May 2000	A	1	A, B	1	B	1	A, B	1	A, B	1
July 2000	A	1, 2	A	1, 2	B	2	A, B	1	A, B	1
September 2000	A	1	A, B	1	A	1	A, B	1	A	1
December 2000			A, B	1	A, B	1	A, B	1		
January 2001			A, B	1	A, B	1	A, B	1	A	1
March 2001			A, B	1			A, B	1	A	1
May 2001	A	1	B	1	B	1	B	1	B	1
July 2001	A	1	A, B	1	B	1	A, B	1	A, B	1
September 2001	A	1, 2	A, B	2	A, B	1, 2	A	1	A, B	2
Cadmium										
February 2000			A, B, C, D	2			A, B, C	1		
March 2000							B, C	1	A, B, C	1
May 2000	B	3	A, B	2, 3	C, D	3	C	1, 2	B, C	1
July 2000	B	2	A	2	B, C	2	A	1	A	1
September 2000	B	2	A, B, C	2	A	1	A	1	A, B	1
December 2000			C, D	2	B, C	1	C	1		
January 2001			B, C, D	2	A, B, C	1	B, C	1	A, B	1
March 2001			B, C, D	2			B, C	1	A, B	1
May 2001	B	1, 2	D	3	D	2, 3	D	1, 2, 3	C	1
July 2001	A	2	A	2	A, B	1, 2	B	1, 2	A, B	1
September 2001	A	2	A	2	A, B	2	A, B	1	A, B	1, 2
Iron										
February 2000			A, B	1			D	1		
March 2000							B, C, D	1	C, D, E	1
May 2000	B, C	1	A	1	C	1	D	1	E	1
July 2000	D	3	B	2, 3	C	2	C, D	1	C, D, E	1
September 2000	C, D	4	A, B	3	B, C	2	A, B, C	1	A, B	1
December 2000			A	3	A, B	2	A	1		
January 2001			A, B	2	A, B	1	A, B, C	1	A, B, C	1
March 2001			A, B	3			A, B	1	B, C, D	2
May 2001	A	2	A, B	3	A, B	2	A, B	1	A	1
July 2001	B	2	B	2	A	1	B, C	1	C, D, E	1
September 2001	B	2, 3	A, B	3	A, B	1	D	1, 2	D, E	1
Lead										
February 2000			B, C	2			A	1		
March 2000							A, B	1	A	1
May 2000	B	2, 3	A, B	1, 2	C, D	3	C	1	B	1
July 2000	B	2	B	2	C	2	B	1	A, B	1
September 2000	C	4	C, D	3	B	2	A, B	1	B	2
December 2000			D, E	3	C, D	2	C	1		
January 2001			D	3	C, D	2	B, C	1	B	1, 2
March 2001			D, E	2			C	1	B	1
May 2001	B	2, 3	E	4	D	3	C	1	B	1, 2
July 2001	A	1, 2	A, B	3	A, B	2	A	1	A	1, 2
September 2001	A	3	A	3	A	2	A	1	A	2

Table 6 (Contd.)

Metal/Date	Puck	Mechelinki	Gdynia	GN Buoy	Vistula plume	
Manganese						
February 2000		B, C, D	2	C, D, E	1	
March 2000				D, E	1	
May 2000	A	2, 3	A, B, C	1, 2	C, D	3
July 2000	A, B	2	A, B, C	2	D	2
September 2000	A, B	4	A, B	3	A	2
December 2000			A, B, C	3	B, C	2
January 2001			B, C, D	3	B, C, D	2
March 2001			C, D	2		
May 2001	A, B	2, 3	D	4	D	3
July 2001	B	1	A, B, C	1	B, C, D	1
September 2001	B	2	A	1, 2	A, B	1
Nickel						
February 2000		B, C	2		A	1
March 2000					A, B	1
May 2000	B	2, 3	B, C	2, 3	D	3
July 2000	A	2	A	2	C	2
September 2000	B	2	A, B, C	2	A	1
December 2000			B, C	3	A, B, C	2
January 2001			A, B	2	A, B	2
March 2001			A, B	3		
May 2001	A	1, 2	B, C	3	A, B, C	2
July 2001	B	3	C	3	B, C	2, 3
September 2001	B	2	B, C	2	C	2

(having allowed for size effects) between sites (Fig. 2). Significant differences are not detectable on every sampling occasion (Table 5), but there is general consistency in the ranking of sites for each metal. In an attempt to produce a single ranking of sites for each metal, only the six sampling occasions with samples from all five sites have been considered. The score (1, 2, 3 etc.) for each site is averaged across the six samples (a score of 2, 3 being recorded as 2.5, a score of 3, 4 as 3.5, etc.). The five sites are then ranked in ascending order of these scores (see Table 7). Thus for copper the rank order of sites in descending order of barnacle accumulated concentrations is Vistula plume, GN Buoy, Gdynia and then Mechelinki and Puck equal. Rank orders of sites for other metals are similarly presented in Table 7. The Vistula plume is also the top ranking site for zinc in the barnacles, while the GN Buoy site leads for cadmium, lead and nickel barnacle concentrations; Gdynia has the highest ranking for barnacle iron concentrations and Puck for manganese (Table 7).

B. improvisus: temporal variation

Accumulated metal concentrations also varied over time for each metal at each site (Table 5), clearly apparent in Fig. 2. It appears that over the total time period (February 2000 to September 2001), zinc and manganese concentrations in barnacles have risen and cadmium concentrations have fallen. Cadmium, lead and manganese barnacle concentrations were relatively

low in the middle of the sampling; those of iron and nickel rose in the middle period (Fig. 2).

M. trossulus: geographical variation

Accumulated metal concentrations in mussels did vary between sites but not on every sampling occasion, especially in the case of zinc (Fig. 3, Table 5). There was nevertheless apparent consistency of the rank orders of sites for each metal between sampling occasions. As for the barnacle data, a single ranking of sites was constructed by averaging the numerical score given in Table 6 across the six sampling occasions when all five sites provided mussel data. The rank orders of sites in descending order of accumulated concentration of each metal in the mussel are given in Table 7. Thus for copper this rank order of sites is GN Buoy, Vistula plume, Gdynia, Mechelinki, then Puck. The GN Buoy site was also the top ranking site for mussel concentrations of zinc, lead, manganese and nickel, while the Vistula plume site led for cadmium and iron mussel concentrations (Table 7).

M. trossulus: temporal variation

Accumulated metal concentrations in mussels also varied over time at each site, although seasonal variation was limited, particularly for Cu and Zn (Table 6). This variation is presented more clearly in Fig. 3. Cadmium also shows little temporal variation; iron, nickel and possibly manganese peak in the middle of the data set, while lead concentrations in the mussels tend to increase over the sampling period (Fig. 3).

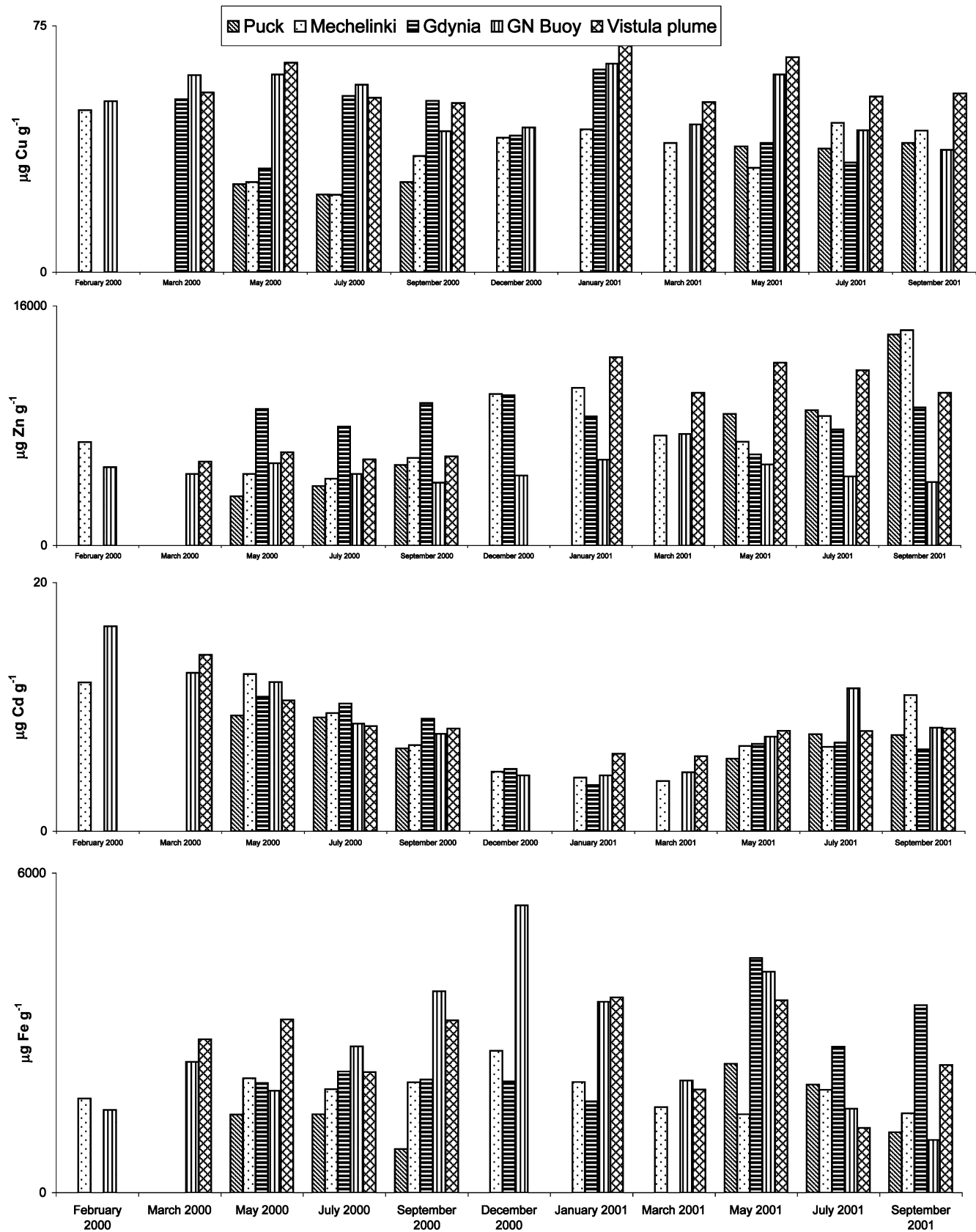


Fig. 2 (Contd.)

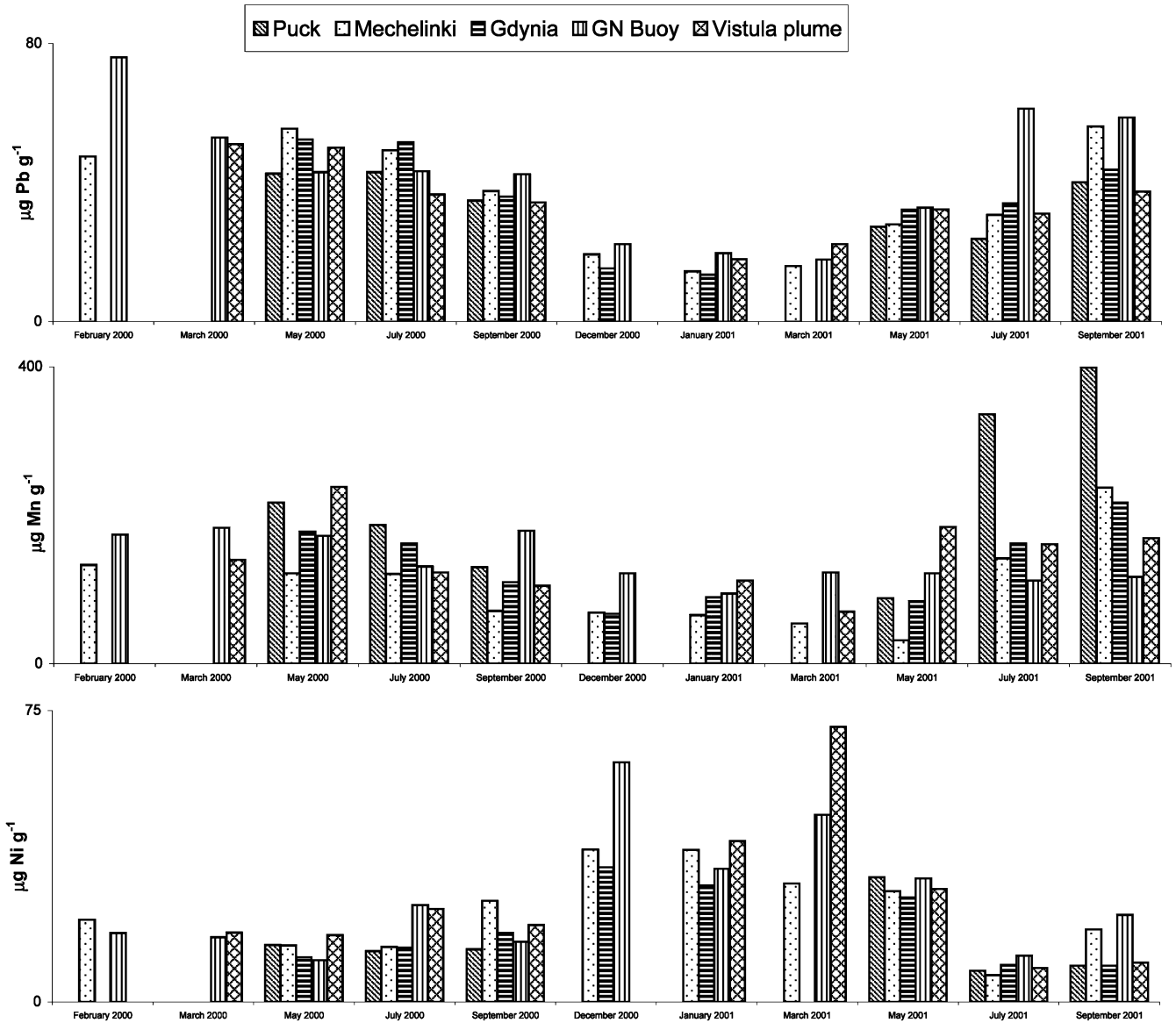


Fig. 2 *B. improvisus*: weight-adjusted mean concentrations of Cu, Zn, Cd, Fe, Pb, Mn and Ni in barnacles collected from five sites on each of up to eleven sampling occasions between February 2000 and September 2001

Correlation of accumulated concentrations in barnacles and mussels

A correlation analysis was employed using the 44 weight-adjusted mean concentrations for the barnacles and mussels from each site (logged data). Figure 4 summarises these correlation analyses for the seven metals. Correlations were significant ($P < 0.05$) for Cu, Zn, Fe, Pb and Ni, but not for Cd and Mn.

Discrimination

Discrimination analysis (again using logged data) was carried out in order to investigate whether the biomonitoring data could discriminate between the individual

sites. As shown in Fig. 5a, four of the five sites could be clearly distinguished when the seven sets of weight-adjusted mean metal concentration data from the two biomonitors were included in the analysis. The Gdynia samples are those that are not clearly demarcated from the others. Results of discrimination analyses using accumulated metal concentration data for barnacles only are shown in Fig. 5b, and for mussels only in Fig. 5c. The barnacle data alone (Fig. 5b) do still allow discrimination of the four sites other than Gdynia but not quite as clearly as the joint data set. The discrimination analysis using mussel data only (Fig. 5c) shows less discrimination than in the case of the barnacle data (Fig. 5b).

Sediments

Data for organic matter contents and ranges of trace metal concentrations in the fraction of surficial sediments smaller than $63\mu\text{m}$ from each site are given in

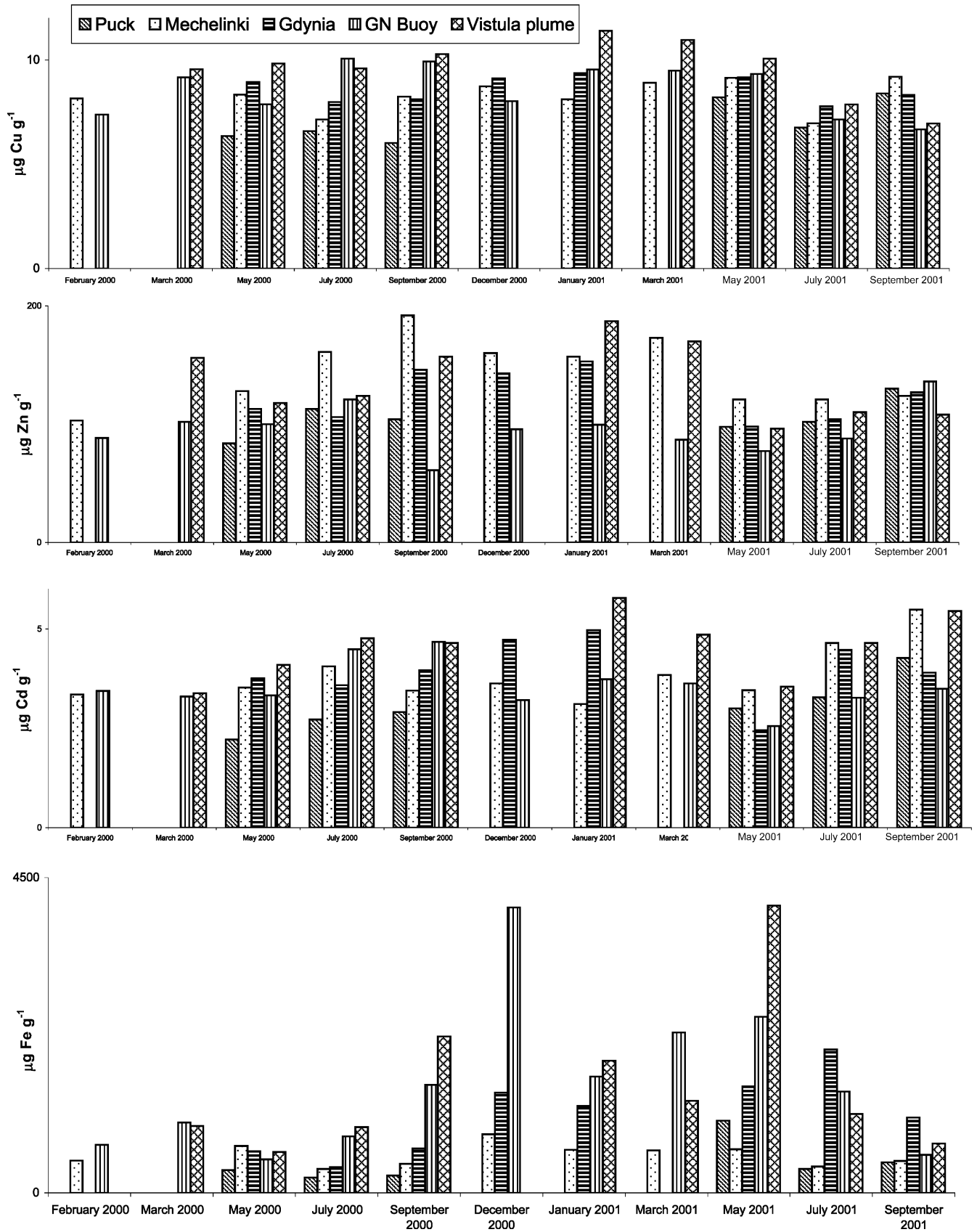


Fig. 3 (Contd.)

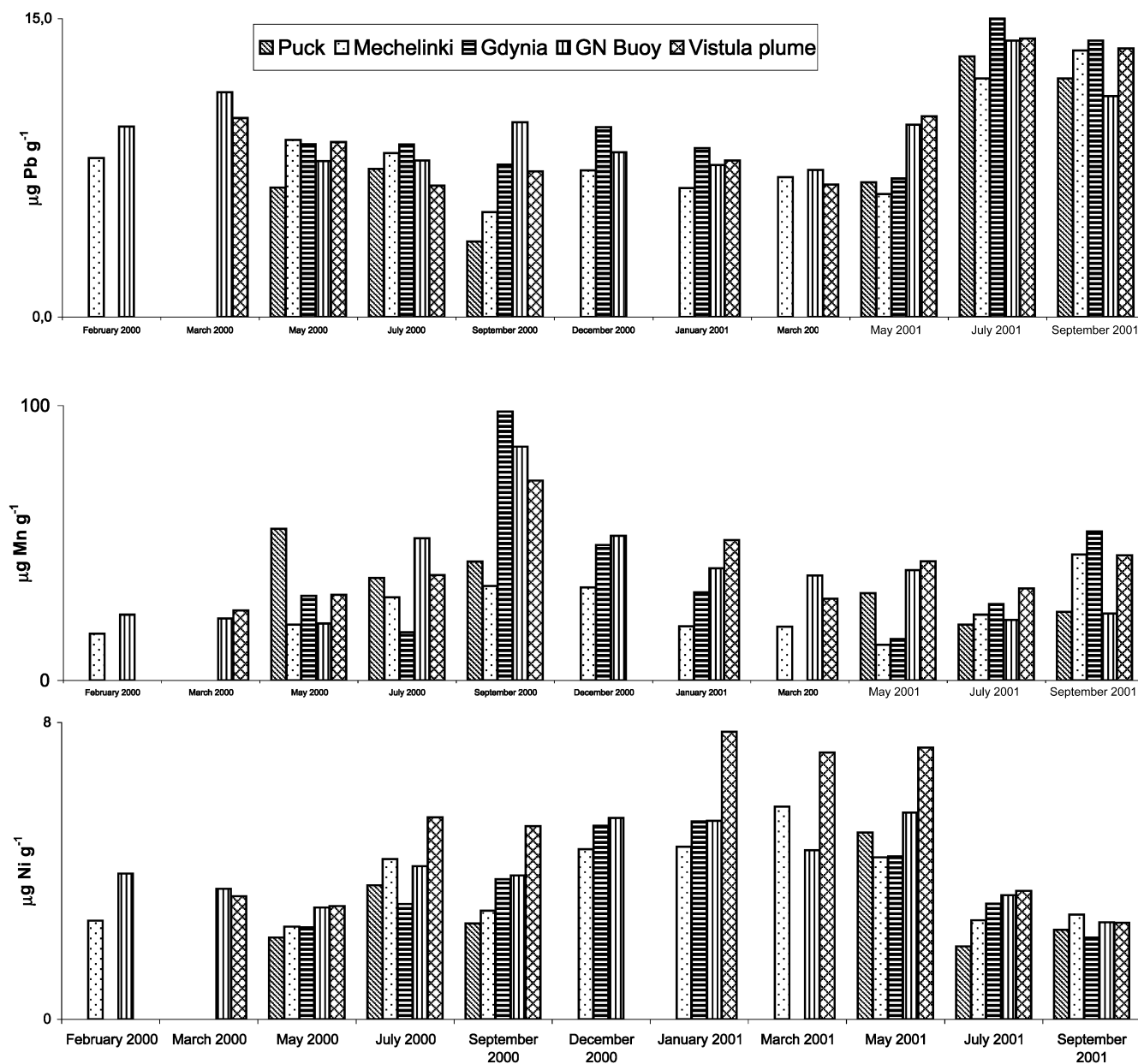


Fig. 3 *M. trossulus*: weight-adjusted mean concentrations of Cu, Zn, Cd, Fe, Pb, Mn and Ni in mussels collected from five sites on each of up to eleven sampling occasions between February 2000 and September 2001

Table 8. The mean metal concentration in the sediment for each site on each sampling occasion was used in correlation analyses with weight-adjusted mean concentrations in either barnacles or mussels (logged data). Table 9 gives the Pearson correlation coefficients of these separate analyses.

Sediment metal concentrations correlated significantly with barnacle accumulated metal concentrations in a single instance, specifically in the case of iron concentrations at Gdynia (Table 9). In the case of the mussels, significant correlation coefficients between sediment and mussel metal concentrations occurred three times, for lead and nickel (inverse correlation) for

the all site data set and for iron (inverse) at Mechelinki. A total of 4 significant results in 84 analyses is to be expected by chance when working at a significance level of $P=0.05$ (particularly when two are positive and two negative), so it is valid to conclude that sediment metal concentrations are not correlated with either barnacle or mussel accumulated metal concentrations in this study.

Discussion

Since accumulated metal concentrations in barnacles and mussels are integrated records of the metals taken up from all sources by that species over a particular previous period, these concentrations are a measure of the recent bioavailability of the metal, summed across all sources,

Table 7 Rank order of sites in descending order of accumulated concentration of each metal in *B. improvisus* and *M. trossulus*

	Puck	Mechelinki	Gdynia	GN Buoy	Vistula plume
Copper					
<i>B. improvisus</i>	4.5	4.5	3	2	1
<i>M. trossulus</i>	5	4	3	1	2
Zinc					
<i>B. improvisus</i>	3	4	2	5	1
<i>M. trossulus</i>	2.5	4.5	4.5	1	2.5
Cadmium					
<i>B. improvisus</i>	3	2	4	1	5
<i>M. trossulus</i>	4	5	3	2	1
Iron					
<i>B. improvisus</i>	5	4	1	2	3
<i>M. trossulus</i>	4.5	4.5	3	2	1
Lead					
<i>B. improvisus</i>	2	3.5	3.5	1	5
<i>M. trossulus</i>	4	5	3	1	2
Manganese					
<i>B. improvisus</i>	1	5	4	3	2
<i>M. trossulus</i>	5	4	3	1	2
Nickel					
<i>B. improvisus</i>	3.5	2	5	1	3.5
<i>M. trossulus</i>	4	5	3	1	2

to that specific biomonitor (Phillips and Rainbow 1994; Rainbow 1995). Accumulated metal concentrations can therefore be used as proxy measures of metal bioavailabilities. The first objective of the study has been met. A data set has been established for the state of trace metal pollution in the Gulf of Gdansk in 2000–2001, as represented by the accumulated concentrations of Cu, Zn, Cd, Fe, Mn, Pb and Ni in the barnacle *B. improvisus* and the mussel *M. trossulus* from five sites.

The data show local geographical differences between the bioavailabilities of all seven metals to either species across the five sites. The Vistula plume and GN Buoy sites were often the sites with the highest trace metal bioavailabilities (Table 7), highlighting the importance of the Vistula river (Fig. 1) as a source of bioavailable metals into the Gulf of Gdansk. The three near-shore sites were usually low in the rankings of bioavailabilities (Table 7), suggesting that local sources of trace metals were less important.

In addition to geographical differences in the bioavailabilities of the seven trace metals, there were also changes in time over the period of sampling. It is clear, therefore, that comparisons against future data sets need to allow for both these local geographical and temporal effects. As an example, it is possible to make a preliminary historical comparison. Rainbow et al. (2000) have provided metal concentration data for *B. improvisus* and *M. trossulus* collected in May 1998 at two of the sites used here, Mechelinki and Gdynia. These data can be compared against those collected in this study in May 2000 and May 2001, as shown for Gdynia data in Table 10. This comparison has been able to identify inter-year differences particularly in the case of the barnacle data, highlighting the power of biomonitoring programmes to follow change over long periods.

Rainbow and Blackmore (2001) have used barnacle data in a similar way to follow changes in trace metal bioavailabilities in Hong Kong coastal waters over more than 10 years.

Rainbow et al. (2000) used comparative mussel data in the literature to conclude that the Gulf of Gdansk has high bioavailabilities (to mussels) of Zn, Cd, Fe and Mn, but not Pb. Since this preliminary study of bioavailabilities in the Gulf of Gdansk, comparative data for *B. improvisus* in the Thames estuary, England have become available (Rainbow et al. 2002). These data were collected in August 2001 and can be compared against barnacle data collected in this study in July 2001 (Table 11). In comparison to the Thames estuary, the Gulf of Gdansk appears to have higher bioavailabilities of Mn, similar bioavailabilities of Cd and Pb, and lower bioavailabilities of Cu, Zn and Fe to *B. improvisus*. Since the Thames estuary was considered to be high in the bioavailabilities of Zn, Cd, Fe, Pb and Mn (Rainbow et al. 2002), it can be concluded that the bioavailabilities of Mn, Cd and Pb to *B. improvisus* in the Gulf of Gdansk should also be considered high on an international scale. It may be worth pointing out here that differences in trace metal bioavailabilities might not result only from differences in total concentrations of metals present, but may be an effect of differences in salinity. Reduced salinity, for example, will increase the uptake of zinc and cadmium from solution by many invertebrates (Rainbow 1997).

A question to be addressed is whether the barnacles and mussels are responding to the same bioavailable sources for particular metals. It is not valid to compare accumulated metal concentrations between species, and certainly not between taxa as far apart

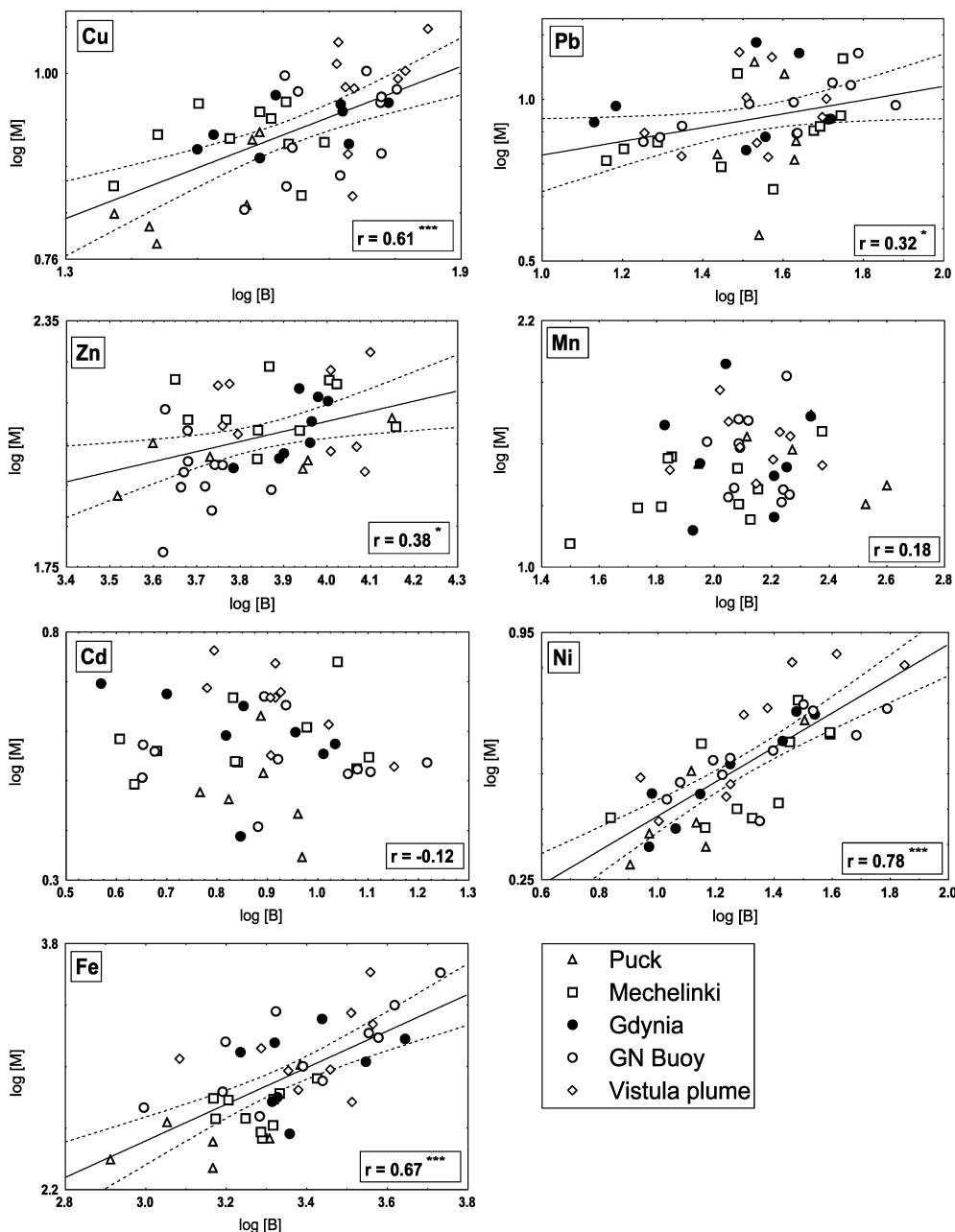


Fig. 4 Correlation analysis between weight-adjusted mean concentrations ($\mu\text{g g}^{-1}$ dry weight, logged) of Cu, Zn, Cd, Fe, Pb, Mn and Ni in barnacles ($\log[B]$) and mussels ($\log[M]$). Regression lines with 95% confidence limits are shown when the correlation coefficient r is significant (* $P < 0.05$, **** $P < 0.001$)

systematically as barnacles and mussels (Rainbow 1995). It is valid, however, to make interspecific comparisons of rank orders of sites ranked in terms of accumulated concentrations, as carried out by Phillips and Rainbow (1988) in a comparative study of barnacles and mussels as trace metal biomonitors in Hong Kong coastal waters, in this case the barnacles *Capitulum mitella*, *Tetraclita squamosa* and *B. amphitrite*, and the mussel *Perna viridis*. Rank orders of

these Hong Kong data showed significant correlations between accumulated concentrations in the barnacles and mussels in the cases of Cu, Zn, Pb and Cr, but not for Cd (Phillips and Rainbow 1988). In the present study, however, the presence of only five sites reduces the power of a rank comparison (Table 7), and a correlation analysis was therefore used to compare weight-adjusted mean metal concentrations in barnacles and mussels collected simultaneously. Accumulated metal concentrations in mussels and barnacles were correlated significantly for Cu, Zn, Fe, Pb and Ni, but not for Cd or Mn (Fig. 4). It is likely therefore that the bioavailable sources of the former five metals to *B. improvisus* and *M. trossulus* are very similar, both from solution and, particularly, from

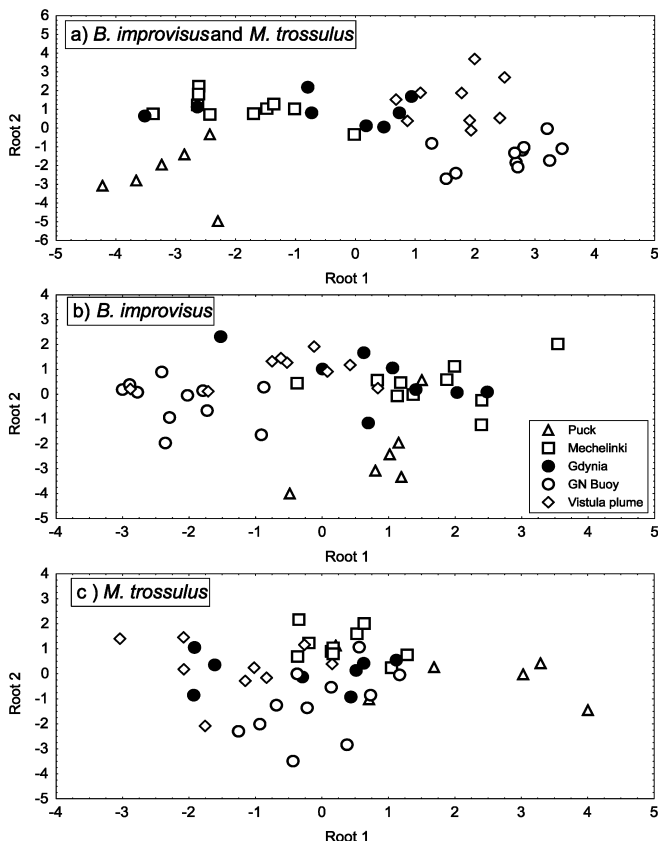


Fig. 5a–c Discrimination analysis of five collection sites using weight-adjusted mean concentrations ($\mu\text{g g}^{-1}$ dry weight, logged) of Cu, Zn, Cd, Fe, Pb, Mn and Ni. **a** In barnacles and mussels combined. **b** In barnacles alone. **c** In mussels alone

food, given the importance of the diet as a source of trace metals to these invertebrates (Wang 2002). The lack of correlation of Cd concentrations in barnacles and mussels in both this study and that of Phillips and Rainbow (1988) may reflect a difference in the relative importance of solution and diet as sources of cadmium for barnacles and mussels. This lack of correlation anyway highlights the importance of using a suite of biomonitors in biomonitoring programmes.

Discrimination analysis indicated that the barnacle is better able to distinguish local differences in trace metal bioavailabilities than the mussel. This is probably related to the relative strength of powers of accumulation, barnacles being particularly strong net accumulators of trace metals (Rainbow 1998, 2002). Mussels also show partial regulation of zinc (and perhaps copper) (Phillips and Rainbow 1988, 1994), reducing their ability to show significant differences in accumulated concentrations between sites not differing greatly in the relevant metal bioavailabilities. If mussels were to show more complete (as opposed to partial) regulation of body concentrations of zinc, then no correlation would be apparent

Table 8 Sediments: ranges of organic matter content (%) and mean trace metal concentrations ($\mu\text{g g}^{-1}$ dry weight, but mg g^{-1} for Fe) in the fraction of surface sediments smaller than $63 \mu\text{m}$ collected from each of five sites on up to 11 sampling occasions between May 2000 and September 2001

	Puck	Mechelinki	Gdynia	GN Buoy	Vistula plume
Organic matter content (%)	6–11	7–23	2–14	4–7	3–7
Copper	6.9–11.7	11.2–29.5	11.3–21.6	7.3–15.1	6.5–13.3
Zinc	38.4–62.0	50.8–93.7	59.8–93.7	38.3–51.9	35.8–89.1
Cadmium	0.51–1.60	0.42–1.49	0.33–1.27	0.05–1.01	0.42–1.20
Iron	2.92–6.02	4.51–10.1	7.6–13.2	4.29–9.55	3.82–10.5
Lead	3.3–13.7	14.1–28.7	27.7–35.7	12.0–20.4	11.4–28.7
Manganese	45.0–243	34.8–575	89.1–261	85.5–256	93.4–465
Nickel	4.0–11.4	2.8–12.3	3.2–10.6	2.4–12.4	2.8–9.5

Table 9 Pearson's correlation coefficients between (logged) metal concentrations in the fraction of sediments smaller than $63 \mu\text{m}$ (mean $\mu\text{g g}^{-1}$ dry weight) and in either barnacles or mussels (weight-adjusted mean, $\mu\text{g g}^{-1}$ dry weight) collected simultaneously from five sites between May 2000 and September 2001. Correlation coefficient is not significant ($P > 0.05$) unless marked with * $P < 0.05$ or ** $P < 0.01$

	All sites ($n = 38$)	Puck ($n = 6$)	Mechelinki ($n = 9$)	Gdynia ($n = 7$)	GN Buoy ($n = 8$)	Vistula plume ($n = 8$)
Copper						
<i>B. improvisus</i>	-0.30	-0.13	-0.46	-0.22	-0.65	0.14
<i>M. trossulus</i>	-0.09	-0.17	-0.39	-0.45	-0.64	0.22
Zinc						
<i>B. improvisus</i>	0.05	-0.21	-0.64	0.14	-0.02	-0.32
<i>M. trossulus</i>	0.32	0.37	-0.15	-0.19	0.09	-0.21
Cadmium						
<i>B. improvisus</i>	0.11	0.58	0.44	0.08	-0.04	0.39
<i>M. trossulus</i>	0.03	-0.05	-0.28	-0.24	0.38	-0.04
Iron						
<i>B. improvisus</i>	0.17	0.08	-0.10	0.84*	-0.20	-0.18
<i>M. trossulus</i>	0.18	0.39	-0.82**	0.35	0.47	-0.40
Lead						
<i>B. improvisus</i>	0.04	-0.49	0.44	0.31	0.13	-0.03
<i>M. trossulus</i>	0.35*	0.54	0.35	0.74	0.66	-0.35
Manganese						
<i>B. improvisus</i>	0.16	0.18	0.51	-0.50	-0.30	0.35
<i>M. trossulus</i>	-0.11	-0.60	0.47	-0.37	-0.31	-0.59
Nickel						
<i>B. improvisus</i>	-0.24	0.08	-0.18	-0.23	-0.15	-0.49
<i>M. trossulus</i>	-0.32*	-0.14	-0.35	-0.16	-0.22	-0.58

Table 10 Inter-year comparisons of weight-adjusted mean metal concentrations ($\mu\text{g g}^{-1}$ dry weight) in barnacles (0.00156 g mean individual dry body weight) and mussels (0.325 g mean individual soft tissue dry weight) collected in May 1998 (after Rainbow et al. 2000), and May 2000 and May 2001 (present study) from Gdynia, the Gulf of Gdansk. Samples sharing the same letter in a row do not differ significantly (ANCOVA, $P > 0.05$) between accumulated metal concentrations in the barnacles or mussels as appropriate

	1998		2000		2001	
	$\mu\text{g g}^{-1}$	Significance	$\mu\text{g g}^{-1}$	Significance	$\mu\text{g g}^{-1}$	Significance
<i>B. improvisus</i>						
Copper	57.2	A	50.5	A	59.1	A
Zinc	9,339	A	8,568	A	5,689	B
Cadmium	14.3	A	9.85	B	6.37	C
Iron	15,450	A	1,965	C	4,184	B
Lead	69.5	A	47.0	B	28.9	C
Manganese	189	A	181	A	85.9	B
Nickel	27.5	A	10.2	B	23.8	A
<i>M. trossulus</i>						
Copper	8.94	A	7.39	A	7.97	A
Zinc	91.0	A	85.8	A	79.5	A
Cadmium	2.56	A	2.84	A	1.83	B
Iron	431	B	513	B	1,319	A
Lead	5.44	A	6.50	A	5.11	A
Manganese	15.4	AB	25.4	A	12.6	B
Nickel	2.58	AB	1.80	B	3.29	A

Table 11 Comparisons of ranges of weight-adjusted mean metal concentrations ($\mu\text{g g}^{-1}$ dry weight) in bodies of *B. improvisus* collected from five sites in the Gulf of Gdansk (this study) and three sites in the Thames estuary (after Rainbow et al. 2002)

	Gulf of Gdansk, July 2001	Thames estuary, August 2001
Copper	37.6–53.5	143–239
Zinc	4,607–11,710	19,040–27,790
Cadmium	6.78–11.49	7.37–9.12
Iron	1,216–2,743	4,870–6,650
Lead	30.7–61.3	26.8–57.9
Manganese	112–336	81.4–96.4

between the accumulated concentrations of zinc in mussels and barnacles.

Sediments also accumulate trace metals from the overlying water and will accumulate them to high concentrations, integrating over time (Rainbow 1995). Sediment metal concentrations are at times used as measurements of metal bioavailabilities, usually after standardisation for the effects of differential organic content and grain size (Phillips and Rainbow 1994). It is relevant, therefore, that metal concentrations in the surficial sediments did not correlate with accumulated concentrations in either biomonitor for any metal. Possible causes for a lack of any relationships would include the different time scales of integration of the metal sources into sediments, barnacles and mussels, and the importance of food as opposed to water as a source of bioavailable metals to barnacles and mussels (Rainbow and Wang 2001; Wang 2002). A conclusion stands out. Sediment trace metal concentrations are not necessarily good proxy measures of ambient metal bioavailabilities to the local filter-feeding invertebrates.

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