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MULTIMETAL ACCUMULATION IN CRUSTACEANS IN SURFACE WATER RELATED TO BODY SIZE AND WATER CHEMISTRY

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Abstract—Many relationships of bioaccumulation of metals have been derived in the past, but verification in the field is often lacking. In the present study, the authors collected field data on bioaccumulation in caged *Daphnia magna* and *Gammarus roeseli* in 12 different contaminated brooks. Besides generating a comprehensive data set on bioaccumulation for these species, the authors also checked whether the bioavailability at the biotic ligand is useful to explain differences in observed bioaccumulation. Increasing bioaccumulation of Mn, Cd, Co, and Ni was observed, which leveled off at higher concentrations. Whole-body concentrations of Ca, Na, Mg, K, Fe, Cu, Se, and Zn were independent of exposure concentrations. Univariate and multivariate regressions were performed to examine the relationships between accumulated metals and dissolved metal concentrations (C_w), fractional occupancy of the biotic ligand (f_{BL}), species weight, and other undefined species traits. Significant relations between body weight and bioaccumulation were found for Na, Fe, Mn, Cd, Co, and Zn; smaller organisms accumulated larger amounts of these elements. Reduced body weight was accompanied by elevated concentrations of Co, Cu, and Fe in *D. magna* and elevated concentrations of Mn in *G. roeseli*, indicating toxicity. Although significant relations were found between bioaccumulation and f_{BL} for Mn and Co, C_w was a better predictor of bioaccumulation. Environ. Toxicol. Chem. 2012;31:2269–2280. © 2012 SETAC

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INTRODUCTION

Bioaccumulation of trace metals in organisms is a natural process that maintains metals at the required physiological level. It is a useful feature when external metal concentrations fluctuate, as is the case under natural field conditions. However, at higher environmental concentrations and for nonessential metals, bioaccumulation can reach critical levels, leading to adverse effects on growth, reproduction, and survival. The ratio between exposure concentrations and whole-body concentrations is the simplest approach to quantify bioaccumulation. As a consequence of essential element regulation, the so-called bioconcentration factors from laboratory studies or bioaccumulation factors from field studies (both further referred to as BCF) are not intrinsic properties of a metal, as is demonstrated by the huge variation of reported BCF values [1].

An important factor that explains some of the variability in accumulated metals is the exposure concentration. For trace elements such as Cd, Cu, Cr, Hg, Ni, Pb, and Zn, BCF values decrease at higher metal concentrations [2,3]. Also, the chemical composition of surface water in terms of hardness, pH, and dissolved organic carbon affects bioaccumulation. These parameters have a large impact on the chemical speciation and bioavailability of metals. It is generally thought that free metal ions are most bioavailable as free metal ions and are transported across cell membranes via protein carriers or membrane channels. In the past decades, biotic ligand models (BLMs) were developed that relate metal toxicity to metals bound to biological receptors. Such models account for the competition between bioavailable metal species and the major cations Ca, Na, and Mg for binding to these receptors [4–6]. The BLMs are useful tools for effect assessment because they relate effects to metal binding to the gill or gill-like organs, which is the primary site of action on the external side of the organism. Following this reasoning, one would hypothesize that the fractional occupancy of the biotic ligand shows stronger relations with accumulated concentrations than with dissolved metals.

Another factor allowing reduction of variability in accumulated metals is size (i.e., body wt or length) of the organism; that is, metal concentrations dramatically increase in small individuals [3,7–9]. Whole-body concentrations roughly consist of two fractions: (1) metals absorbed to the internal body and (2) metals adsorbed to the body surface. The surface area to volume ratio is relatively high for small organisms. Whereas metals adsorbed to the external body surface may be a negligible fraction in larger animals, they take an increasingly larger proportion of total-body burden in small animals.

Due to chemical similarity, some metals may share uptake pathways, for example, Zn and Cd, and interactions between metals are to be expected. A meta-analysis [10] showed that in the majority of studies effects of metal mixtures are not simply additive. Synergistic as well as antagonistic effects are found and depend on the metal combination and the type of end point (i.e., accumulation, growth, reproduction, or mortality) studied. A clear general pattern of interaction effects was not observed.

Because mixture patterns are ambiguous in many laboratory studies, we focused our research on bioaccumulation of metals in caged crustaceans in diffusely contaminated natural surface waters. Fish and bivalves have been tested in situ for bioaccumulation, but field data for smaller invertebrates are scarce [11,12]. Standardized laboratory tests often underestimate the

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risks of metals in terms of accumulated amounts [13]. The ecological relevance and robustness of field data are high because of the realistic exposure conditions, the high variation in chemical composition and other environmental conditions among sites [14].

The European Water Framework Directive (Point 1.2.6. of Annex V) states that laboratory data on persistence and bioaccumulation always should be compared with evidence from field studies (http://ec.europa.eu/environment/water/water-framework/). Because field data on bioaccumulation are scarce, available only for a limited number of metals and hardly available for freshwater crustaceans, our aim was to fill this gap by exposing caged organisms to natural surface waters, contaminated with a cocktail of metals and metalloids. The advantage of caged organisms is that the origin, life stage, and exposure time of the organisms are controlled, while exposure conditions reflect realistic conditions well.

The aims of our study were as follows: (1) to derive model parameters for the relation between exposure concentration and whole-body concentrations and BCF for common freshwater crustaceans for a broad group of metals and metalloids under field conditions; (2) to test whether fractional occupancy of the biotic ligand is a better predictor of bioaccumulation than dissolved concentrations; and (3) to assess inter- and intraspecific size-dependent bioaccumulation.

METHODS

General setup

We exposed the filter feeder *Daphnia magna* and the shredder *Gammarus roeseli* in situ to ambient field concentrations in 12 diffusely contaminated small rivers in seminatural areas in The Netherlands, and measured the accumulation of 12 common, essential, and nonessential elements. We chose *D. magna* because it is the most common invertebrate laboratory test organism in toxicity experiments. It is a filter feeder, and individuals of our clone reach a size of approximately 5 mm under optimal laboratory conditions. In Dutch surface waters, *D. magna* is not abundant, though the related *D. pulex* is quite common. We selected *G. roeseli* because of its ecological relevance; it is widespread and abundant in Dutch rivers and plays a vital role in the food chain. It is an omnivorous species that shreds leaves and debris and eats smaller invertebrates but also constitutes an important food source for fish and birds. Male adult *G. roeseli* can reach a size of approximately 3 cm; females are smaller.

Accumulation of Ca, Na, Mg, K, Fe, Mn, Cd, Co, Cu, Ni, Se, and Zn was measured. These 12 elements are natural constituents of surface waters, but may also occur at elevated concentrations due to anthropogenic activities (traffic, agriculture, industrial and domestic wastewater, mining). Except for Cd, these elements are essential for basic cellular functions such as maintenance of osmotic pressure. The presence of Ca, Na, Mg, and K is known to affect the bioavailability of Co, Cd, Cu, Mn, Ni, and Zn. Whereas most of these metals mostly occur as cations under ambient conditions, Se is a metalloid that occurs as an oxyanion, predominantly SeO_4^{2-}. The elements Fe and Mn were included because of their role in cellular respiration (oxygen binding and redox conditions).

Site selection

Twelve upstream small rivers on sandy soils were selected in the Dommel catchment area in The Netherlands (Fig. 1). The Dommel rises in the northeast of Belgium and runs north through the southeast of The Netherlands until it joins the Meuse. The upstream part of the Dommel is heavily affected by ongoing zinc-smelting activities dating back to 1904, industrial and municipal wastewater inflow, and leaching and runoff from agricultural land [15]. A natural process contributing to elevated concentrations of Ni and Co is pyrite oxidation enhanced by excessive nitrate from agricultural practice [16]. The area is shown to exceed water type–specific environmental quality criteria up to a factor of 100, based on generic quality criteria.

Site selection was based on evaluation of monitoring data over 2007 to 2009, calculated risks for Cu, Ni, and Zn [17], geographical considerations, and visual inspection of candidate sites. The following selection criteria were applied: (1) selected sites cover a range of low to high metal exposure, (2) the pH values of the sites are within the tolerance range of *D. magna*, (3) only natural streams are selected, and (4) they are as far from urbanization as possible.

Selected rivers were Beekloop, Beerze, Boschloop, Dommel, Gender, Groote Beerze, Groote Waterloop, Keersop, Reusel, Run, Tongelreep, and Tungelroyse Beek.

Daphnia magna *field experiment*

The *D. magna* clone K4 was cultured in our own laboratory at 20°C in M4 medium, according to the Organisation for Economic Co-operation and Development (OECD) 211 protocol [18]. Four cages were installed on each site, consisting of an inner and an outer cage. The inner cage was made of acrylic glass (methylmethacrylate), was 20 cm high and 9 cm in diameter, and had a filter area of approximately 200 cm^2, covered with Monodur PA 500N (500-μm mesh) gauze. In a lab test it was confirmed that neonates were unable to escape from the cage with 500-μm mesh. The outer cage was applied for protective reasons and was made of steel with 1-mm steel mesh, 21 cm high and 11 cm in diameter. Cages were installed approximately 10 cm below the water level.

On June 9 and 29, 2010, respectively, 20 and 10 *D. magna* (<2 d old) were put in the cages. After three weeks of exposure, daphnids were collected from the field and brought to the lab. Adult and juvenile daphnids were separated and transferred to M4 medium for overnight depuration at 15°C. Adults and juveniles were stored in separate Eppendorf tubes, freeze-dried, and kept at −18°C.

Gammarus roeseli *field experiment*

Adult *G. roeseli* were sampled June 2, 2010 from the Tongelreep and transferred to the lab, where they were kept in breeding chambers in glass aquaria with 50% Tongelreep water and 50% M4 medium, under aeration at 20°C and a 12-h light/dark regime. Gammarids were fed ad libitum with dried leaves (*Populus tremula*) taken from the site, and juvenile *D. magna* from our lab culture were supplied as an additional food source once a week. Each week, half of the growth medium was replaced with fresh M4 medium and juveniles were separated from the parents. New batches of *G. roeseli* were started with juveniles. A field experiment was set up with juveniles born between June 21 and 27, 2010. Individual juvenile gammarids were transferred to 100-ml polyethylene vessels with 250-μm nylon (Monodur PA 250N) mesh in the screw cap. The exchange surface was 3.5 cm in diameter. Eight vessels, containing 50 to 100 mg dried leaves and one gammarid per vessel, were installed on June 29, on the same sites as the *D. magna* trials. After three weeks, gammarids were transferred to the lab in their (original) vessels, taken out the next day, stored in

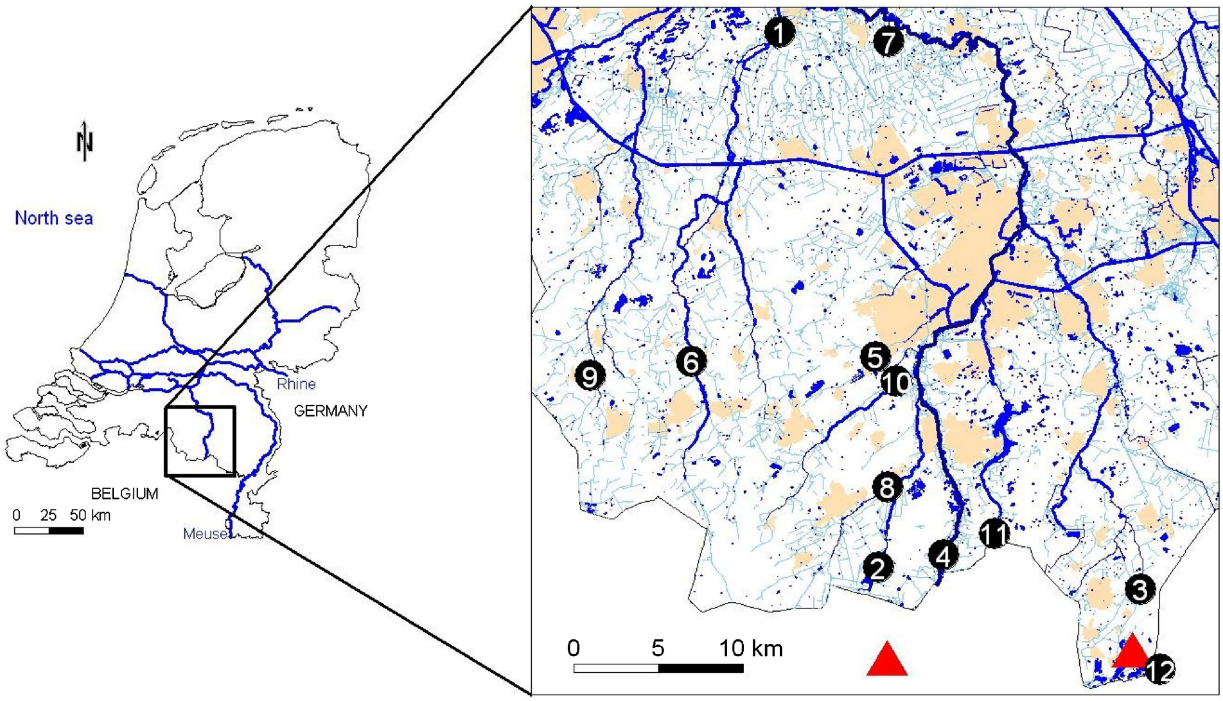

Fig. 1. Map of The Netherlands (left) with Dommel tributary test region (square). The detailed map (right) shows rivers, brooks, lakes, fens, and canals. Triangles indicate location of zinc manufacturers. Trial sites: 1 = Beerze; 2 = Beekloop; 3 = Boschloop; 4 = Dommel; 5 = Gender; 6 = Groote Beerze; 7 = Groote Waterloop; 8 = Keersop; 9 = Reusel; 10 = Run; 11 = Tongelreep; 12 = Tungelroyse Beek. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com]

separate Eppendorf tubes, freeze-dried, and kept at –18°C until further analysis.

Water chemistry

At the start and the end of the experiment, the following parameters were determined on site: pH, water temperature, O_2 saturation, and conductivity. Water samples were taken for dissolved organic carbon (DOC) and metal analysis in the laboratory. After filtration (0.22 μm) and acidification, Na, Ca, Mg, Mn, Fe, K, Cd, Co, Cu, Ni, Se, and Zn were analyzed by high-resolution inductively coupled plasma-mass spectrometry (ICP-MS). These measurements were supplemented with surveillance monitoring data from the water-board authority from the same period as the field exposure took place. Average concentrations in water and variation coefficients ([standard deviation/mean] × 100) over the test period were calculated, based on a total of four to five measurements.

Bioaccumulation

Harvested biomass was divided or pooled to obtain samples of approximately 500 μg dry weight. Metal accumulation was determined by acid destruction of at least 100 μg freeze-dried biomass per vial with 2 ml HNO_3 (Ultrapure) at 120°C. Blank controls (empty vials as well as daphnids grown in M4 medium) were also subjected to the destruction procedure. The residue was dissolved in 3 ml 9% (v/v) HNO_3. Standard samples of dogfish liver were analyzed as a control. After every tenth sample, two analytical blanks were measured. Detection limits in dissolved destruate were 80 μg Ca/L, 0.40 μg Cd/L, 0.30 μg Co/L, 0.6 μg Cu/L, 8 μg Fe/L, 40 μg K/L, 30 μg Mg/L, 0.9 μg Mn/L, 60 μg Na/L, 0.5 μg Ni/L, 1 μg Se/L, and 1 μg Zn/L.

Analysis of these small amounts of biomass is sensitive for contamination during sampling and sample destruction. Outliers were characterized by extremely high Zn concentrations (>1,100 μg/g dry wt) when these values are not confirmed by duplicate samples. Outliers were removed from the data set prior to statistical analysis of the data. It concerned six out of 24 samples of *G. roeseli* and three out of 52 samples of *D. magna* adults.

Modeling

The software WHAM7 was used to compute speciation for Cd, Co, Cu, Mn, Ni, and Zn; the major competing cations Ca, Na, Mg, and K; and the major anorganic ligands Cl, SO_4, OH, and CO_3. Calculated free ion activities are provided in Supplementary Data, Table S1. The fractional occupation of the biotic ligand (f_{BL}) was computed by

$$f_{BL} = \frac{K_{Me\text{-}BL} \times \alpha_{Me}}{1 + K_{Me\text{-}BL} \times \alpha_{Me} + \sum K_{i-BL} \times \alpha_i} \quad (1)$$

where K is the affinity of the metal for the biotic ligand, α is the free ion activity, Me is the metal, and i represents competitive ions Ca, Mg, Na, and/or H [19]. Because $CuOH^+$ and $CuCO_3$ are known to contribute to Cu toxicity, $K_{Me\text{-}BL} \times \alpha_{Me}$ in the nominator and denominator is replaced by $K_{Cu\text{-}BL} \times \alpha_{Cu} + K_{CuOH\text{-}BL} \times \alpha_{CuOH} + K_{CuCO3\text{-}BL} \times \alpha_{CuCO3}$ to describe Cu binding to biotic ligands, according to De Schamphelaere and Janssen [19]. Affinity constants were obtained from the literature (see Table 1). For Co, no data were available for binding to crustacea; therefore, we used $\log K_{BL}$ obtained for the fish *Oncorhynchus mykiss*. It is assumed that affinity constants for metal binding to the biotic ligand can be applied across different species. In cases where both acute and chronic data were available, we selected chronic data.

Bioaccumulation was described by a simple empirical model [2,3]

$$C_b = a \times C_w^b \quad (2)$$

Table 1. Affinity constants for binding of metals and macro-ions to the biotic ligand ($\log K_{BL}$) and to fulvic acids ($\log K_{FA}$)

	Me = Cd	Me = Co	Me = Cu	Me = Mn	Me = Ni	Me = Zn
	Daphnia pulex	*Oncorhynchus mykiss*	*Daphnia magna*	*Ceriodaphnia dubia*	*Daphnia magna*	*Daphnia magna*
Reference	[28]	[32]	[5]	[31]	[33]	[34]
$\log K_{BL}$						
Me	7.0	5.1	8.02	2.23	4.0	5.31
MeOH			8.02			
$MeCO_3$			7.44			
H	6.1	6.2	6.67			5.77
Ca	4.1	4.7		3.07	3.10	3.22
Na		3.2	2.91			1.90
Mg	3.7			3.08	2.47	2.69
$\log K_{FA}$	1.51	1.35	2.17	1.76	1.43	1.68

or as a BCF

$$\mathrm{BCF} = a \times C_w^{b'} \quad (3)$$

in which $b' = b - 1$. In this model, the accumulated metal (C_b) is proportional to the metal concentration in water (C_w) raised to the power b. If $b = 0$, whole-body concentrations are constant, reflecting regulation by organisms. If $b = 1$, internal body concentrations are proportionally related to external concentrations, reflecting the absence or failing of a regulatory system. Usually b is less than 1, which implies that BCFs ($\mathrm{BCF} = C_b/C_w$) decrease at higher metal concentrations. To test whether fractional occupation of the biotic ligand is a better descriptor of bioaccumulation, we replaced C_w in Equations 2 and 3 by f_{BL}.

The relationship between bioaccumulation and body size is described by an allometric equation

$$C_b = a \times W^{\kappa} \quad (4)$$

In this model, whole-body concentration (W) is proportional to the metal concentration in water raised to the power κ. Parameters a and κ are fitting constants. The factor C_b is independent of species weight when $\kappa = 0$ and inversely related to species weight when $\kappa = -1$. Model parameters in equations 2, 3, and 4 were derived by linear regression of the log-transformed equations for *D. magna* and *G. roeseli* separately (intraspecies relations) and for *D. magna* and *G. roeseli* together (interspecies relations).

Models that describe whole-body concentrations as a combined function of exposure concentrations and size were derived by regression after log transformation of

$$C_b = a \times C_w^{b} \times W^{\kappa} \quad (5)$$

Additionally, it was tested whether inclusion of species as a binary covariable resulted in a significant improvement of the regression. If species is a significant covariable, a correction factor for *Gammarus* compared to *Daphnia* follows from the regression

$$\log(C_b) = \log(a) + b \times \log(C_w) + \kappa \times \log(W) + \lambda \times \mathrm{Sp} \quad (6)$$

where Sp represents the presence of a particular species. For *D. magna* Sp = 0, and for *G. roeseli* Sp = 1, and λ is the correction factor for the effect of species on bioaccumulation.

Based on Equation 6, 12 candidate models were fitted to the data: (1) $C_b \sim$ constant, (2) $C_b \sim C_w$, (3) $C_b \sim W$, (4) $C_b \sim \mathrm{Sp}$, (5) $C_b \sim C_w \times W$, (6) $C_b \sim C_w \times \mathrm{Sp}$, (7) $C_b \sim W \times \mathrm{Sp}$, (8) $C_b \sim C_w \times W \times \mathrm{Sp}$, (9) $C_b \sim f_{BL}$, (10) $C_b \sim f_{BL} \times W$, (11) $C_b \sim f_{BL} \times \mathrm{Sp}$, and (12) $C_b \sim f_{BL} \times W \times \mathrm{Sp}$. The same models are tested for BCF. The best regression model for each metal was selected based on the Akaike information criterion (AIC) [20]. In general, model selection keeps a balance between predictive power and desired model simplicity. Theoretically, the model with the lowest AIC is the best. However, to prevent overfitting, a statistical penalty is given based on the number of parameters; that is, a three-parameter model should be at least two AIC units lower than the simpler two-parameter model to be selected. Additionally, the p value must be <0.05 to be selected as the best model. The adjusted r^2 is less suitable for model selection because leverage and skewness of the data distribution can lead to an increased adjusted r^2, which does not reflect a real model improvement. All statistical analyses were performed in R, version 2.14.0 (R Foundation for Statistical Computing, http://cran.r-project.org/).

RESULTS

Water chemistry

The mean water chemistry of trial sites, aggregated over a two-month test period, is given in Table 2. Concentrations are variable in time, with variation coefficients mostly between 10 and 60, but with peaks up to >100. Several of the selected sites exceed environmental quality criteria (EQS), composed of maximum admissible concentrations plus background concentration: $0.9 + 0\,\mu g$ Cd/L (specific value for hardness, 100–200 mg $CaCO_3$/L), $1.36 + 0.2\,\mu g$ Co/L, $1.1 + 0.4\,\mu g$ Cu/L, $20\,\mu g$ Ni/L, $24.6 + 0.04\,\mu g$ Se/L, and $15.6 + 1\,\mu g$ Zn/L (www.rivm.nl/rvs/normen). Criteria for chloride and sulfate are 200 and 100 µg/L, respectively. The sites Beekloop, Grote Waterloop, and Tongelreep are the cleanest sites, with none or only slight exceedances, whereas Run and Gender exceed criteria for Co, Cu, Ni, and Zn. Dommel and Tungelroyse Beek also constitute high concentrations of Zn but add relatively high concentrations of Na, Se, Cl, and SO_4. Copper EQS is exceeded at 10 out of 12 sites; however, DOC might reduce the effects of Cu significantly, especially on sites with DOC >5 mg/L.

Maximum risk ratios (concentration/EQS) are Zn 5.5 in Dommel, Ni 4.5 in Run, Cu 2.5 in Keersop, Co 18 in Run. Most risk ratios are a factor of 2 to 3 higher when concentrations are related to average annual EQS. An exception is Se, which exceeds annual average EQS at eight sites, up to a factor of 50 in Dommel and Tungelroyse Beek.

Survival of test organisms

The amount of organisms that could be collected after three weeks of field exposure differed between sites. The total destructed (collected) biomass is given in Table 3. In Boschloop and Dommel, only a small amount of adult daphnids could be harvested, no juveniles were present, and *G. roeseli* did not

Table 2. Characteristics of filtered water samples of selected trial sites: mean and variation coefficient (%) over June and July 2010[a]

		mg/L										μg/L					
Site	pH	DOC	Cl	SO_4	HCO_3	Ca	Na	Mg	K	Fe	Mn	Cd	Co	Cu	Ni	Se	Zn
BEE	7.4^{1}	12^{6}	34^{27}	75^{6}	84^{20}	40^{10}	26^{21}	6.4^{8}	11^{18}	0.47^{59}	0.3^{27}	0.04^{29}	3.3^{34}	3.4^{94}	15^{9}	0.022^{107}	8.5^{59}
BEL	7.3^{1}	5.8^{9}	38^{6}	50^{16}	140^{10}	51^{2}	28^{4}	6.9^{6}	5.7^{13}	0.21^{15}	0.08^{20}	0.06^{22}	2.1^{20}	1.6^{78}	6.6^{7}	0.15^{50}	14^{45}
BOL	7.7^{14}	9.2^{8}	26^{33}	46^{11}	49^{92}	36^{39}	23^{30}	5.5^{32}	4.7^{28}	1.6^{128}	0.06^{82}	0.10^{80}	0.25^{5}	3.1^{62}	2.9^{15}	0.21^{70}	41^{98}
DOM	7.2^{1}	6.5^{4}	190^{52}	175^{53}	140^{0}	35^{4}	130^{36}	6.2^{15}	100^{48}	0.11^{56}	0.05^{50}	0.70^{33}	2.1^{33}	2.6^{42}	11^{13}	2.9^{31}	91^{23}
GEN	6.6^{2}	3.2^{27}	30^{0}	83^{2}	48^{19}	32^{6}	23^{4}	8.0^{5}	6.2^{8}	0.66^{123}	0.26^{48}	0.20^{34}	18^{82}	1.9^{75}	52^{22}	0.048^{231}	43^{42}
GRB	7.2^{1}	10^{7}	49^{9}	85^{1}	90^{7}	43^{13}	43^{11}	6.3^{8}	17^{17}	0.3^{52}	0.28^{23}	0.06^{31}	6.0^{35}	2.1^{80}	15^{19}	0.06^{44}	19^{68}
GRW	8.2^{11}	6.0^{5}	39^{0}	47^{5}	150^{9}	58^{11}	23^{16}	6.7^{17}	3.0^{78}	0.25^{22}	0.13^{41}	0.04^{44}	0.34^{22}	1.3^{112}	1.4^{23}	0.06^{50}	5.4^{73}
KSP	7.2^{2}	9.4^{3}	27^{10}	63^{6}	74^{24}	39^{6}	20^{11}	6.2^{8}	9.8^{6}	0.12^{47}	0.05^{77}	0.09^{57}	1.9^{71}	3.8^{31}	11^{29}	0.22^{19}	25^{58}
REU	6.5	30	39	130	110	47^{11}	22^{11}	9.9^{15}	16^{47}	4.2^{185}	0.25^{87}	0.03^{44}	3.7^{57}	2.9^{68}	12^{38}	0.079^{43}	18^{75}
RUN	6.8^{3}	7.7^{9}	24^{9}	110^{0}	36^{22}	35^{8}	16^{5}	8.5^{17}	12^{25}	0.33^{49}	0.14^{86}	0.08^{59}	24^{127}	3.3^{31}	91^{37}	0.42^{30}	35^{81}
TOR	7.3^{13}	4.2^{12}	45^{5}	63^{3}	150^{0}	54^{19}	32^{25}	7^{21}	6.0^{25}	0.06^{62}	0.03^{50}	0.04^{28}	0.88^{22}	2.0^{60}	5.4^{12}	0.28^{34}	7.7^{87}
TUB	7.1^{3}	5.5^{14}	355^{12}	396^{3}	189^{3}	95^{9}	250	16^{20}	9.1	0.05^{39}	1.0^{81}	0.06^{67}	0.3^{55}	1.4^{84}	3.0^{35}	2.7^{NA}	32^{41}

[a] Superscript numbers express variation coefficient (%).
DOC = dissolved organic carbon; BEE = Beerze; BEL = Beekloop; BOL = Boschloop; DOM = Dommel; GEN = Gender; GRB = Groote Beerze; GRW = Groote Waterloop; KSP = Keersop; REU = Reusel; RUN = Run; TOR = Tongelreep; TUB = Tungelroyse Beek.

survive. Also, in Keersop and Tungelroyse Beek, reproduction of *D. magna* failed. Reproduction of *G. roeseli* was not expected due to its young age. Whole-body concentrations are also expressed in Table 3. Due to the complex mixtures of elevated trace-metal concentrations, it is difficult to establish causal relationships between individual elements and observed effects on survival and reproduction. The data set, however, offers the opportunity to assess relationships between external exposure and accumulation of a large number of elements in a natural complex environment in two different species and to compare them with background accumulation in a standard M4 growth medium.

Body concentrations

Field-exposed daphnids accumulate larger amounts of all metals except Ca compared to the control daphnids grown in M4 medium in the lab. Although Ca concentrations in the field are higher than in M4 medium, daphnids keep Ca constant at approximately 8%, which is normal in *D. magna* [21]. Whole-body concentrations of Co, Cd, Fe, and Ni are a factor of 20 to 30 higher; Na and Mn are a factor of 8 to 10 higher; and Mg, K, Cu, Se, and Zn are a factor of 2 to 3 higher in field-exposed adult daphnids compared to the control. The elements Cd and Se were also detected in control daphnids, whereas they are not con-

Table 3. Whole-body residues of macro-ions and trace elements in *D. magna and G. roeseli* after 3 weeks of field exposure[a]

			Ca	Na	Mg	K	Fe	Mn	Cd	Co	Cu	Ni	Se	Zn
	n	Biomass μg	mg/g dry wt						μg/g dry wt					
Daphnia magna														
Control	6	400	84^{10}	6.8^{149}	1.1^{56}	5.2^{46}	0.7^{69}	0.12^{86}	0.19^{40}	0.85^{75}	31^{37}	2^{29}	0.6^{30}	98^{5}
BEE	9	552	60^{38}	89^{59}	2.7^{35}	11^{37}	13^{57}	0.78^{62}	1.3^{154}	18^{52}	76^{28}	130^{147}	1.4^{73}	230^{97}
BEL	4	875	80^{4}	50^{48}	2.6^{21}	9.1^{24}	18^{31}	0.26^{25}	1.3^{8}	12^{27}	110^{17}	210^{163}	2.3^{22}	250^{41}
BOL	1	569	67	37	2.1	8.8	2.1	0.053	1.0	0.78	42	29	0.53	140
DOM	1	144	67	92	2.5	13	12	0.14	20	4.1	130	3.9	5.8	260
GEN	4	404	77^{35}	38^{44}	2.1^{28}	8^{16}	44^{42}	0.57^{29}	2.8^{27}	65^{41}	110^{57}	49^{73}	2.7^{51}	190^{25}
GRB	6	535	69^{18}	34^{90}	2^{27}	7.3^{31}	41^{32}	0.47^{48}	0.74^{24}	19^{35}	92^{25}	10^{32}	2.8^{30}	240^{68}
GRW[c]	5	423	97^{23}	60^{82}	3.8^{27}	11^{46}	17^{29}	0.87^{28}	0.65^{34}	4.4^{39}	95^{84}	17^{134}	1.4^{37}	180^{17}
KSP[b]	5	372	75^{69}	19^{98}	1.3^{62}	5^{51}	19^{64}	0.44^{89}	1.8^{69}	22^{87}	110^{22}	56^{168}	2.1^{14}	160^{44}
REU	1	252	88	130	4.2	14	57	0.3	1.1	12	250	15	2.9	120
RUN	2	476	85^{7}	66^{76}	2.5^{31}	9.2^{23}	18^{14}	0.45^{97}	1.3^{38}	26^{33}	84^{17}	140^{102}	1.2^{34}	160^{2}
TOR	4	626	89^{14}	74^{55}	3.1^{28}	11^{30}	12^{24}	0.23^{8}	2.1^{21}	9.8^{17}	73^{40}	18^{92}	2.3^{42}	230^{16}
TUB	4	326	76^{22}	120^{56}	2.9^{29}	12^{29}	5.8^{30}	5.2^{28}	8.9^{79}	5.7^{14}	110^{14}	8.8^{123}	1.3^{72}	520^{26}
Mean			78^{14}	67^{52}	2.7^{30}	10^{26}	22^{77}	0.81^{172}	3.6^{157}	17^{103}	107^{47}	57^{116}	2.2^{60}	223^{47}
Gammarus roeselli														
BEE	4	243	100^{6}	20^{169}	2.2^{48}	10^{27}	0.81^{113}	0.19^{133}	0.36^{42}	2.4^{137}	120^{34}	26^{131}	1.9^{124}	140^{61}
GEN[b]	2	125	93	100	4.3	13	3.3	0.26	2.2	4.5	90	6.4	0.96	69
GRB	3	420	110^{10}	24^{85}	2.4^{6}	11^{16}	2.2^{102}	0.2^{75}	0.29^{14}	2.4^{81}	130^{20}	79^{168}	3^{103}	81^{19}
KSP	3	627	95^{22}	0.15^{29}	2^{13}	7.7^{18}	1.3^{62}	0.12^{88}	1.5^{15}	2.2^{43}	110^{7}	1.3^{29}	0.99^{87}	58^{5}
REU	2	537	85^{41}	27^{63}	3.1^{42}	9.3^{52}	1.4^{66}	0.084^{23}	0.31^{21}	2^{62}	83^{31}	1.5^{42}	0.3^{3}	53^{57}
RUN[c]	5	316	120^{1}	12^{129}	2.6^{27}	9.9^{25}	2.1^{32}	0.24^{42}	0.66^{13}	23^{37}	110^{13}	4.1^{131}	2.2^{23}	340^{72}
TUB	2	333	110	0.73	2.4	8.5	1.1	0.20	0.81	1.9	150	2.1	3.6	110
Mean	0		102^{12}	26^{130}	2.7^{29}	10^{17}	1.7^{49}	0.18^{34}	0.9^{82}	5.5^{142}	113^{20}	17^{166}	1.9^{64}	122^{83}

[a] Superscript numbers express variation coefficient (%),
[b] One outlier removed from the dataset.
[c] Two outliers removed from the dataset.
n = number of destruction replicates; Biomass = total destructed material; BEE = Beerze; BEL = Beekloop; BOL = Boschloop; DOM = Dommel; GEN = Gender; GRB = Groote Beerze; GRW = Groote Waterloop; KSP = Keersop; REU = Reusel; RUN = Run; TOR = Tongelreep; TUB = Tungelroyse Beek.

stituents of M4 medium. The elements Cd and Se could have been present as impurities in trace element or vitamin stock solutions, or they could have entered via the algae fed to the daphnids.

Accumulation in *D. magna* could be compared with accumulation in *G. roeseli* at seven sites. *Gammarus roeseli s*howed significantly higher Ca content and significantly lower content of Fe and Co. Remarkably lower whole-body concentrations of Na, Mn, Cd, and Ni were found in *G. roeseli*; but in a paired *t* test, these differences were not significant. More observations at more sites are required to confirm these findings.

Dissolved concentrations

The relationship between whole-body concentrations, corresponding BCF values, and aqueous concentrations in *D. magna* and *G. roeseli* is shown in Figure 2. Three elements clearly come forward for which whole-body concentrations depend on dissolved concentrations in one or more of the tested combinations of species and chemical forms: Mn, Cd, and Co. Whole-body concentrations of the other elements (Ca, Na, Mg, K, Fe, Cu, Ni, Se, and Zn) show no significant relation with exposure, implying that the whole-body concentrations of these elements are constant. In *G. roeseli,* relations were found only between whole-body concentrations and dissolved Cd.

Occupancy of the biotic ligand

The occupancy of the biotic ligand is affected by competition between free ions. According to Equation 1, two parameters are decisive in the ultimate ligand binding of a metal: (1) the level of the affinity constant, and (2) the concentration of the metal—both compared to the affinity constants and concentrations of its competitors.

Linear regression between $\log(C_b)$ and $\log(f_{BL})$ revealed significant relations for Mn (slope $= 0.78 \pm 0.25$, $r^2 = 0.35$) and Co (slope $= 0.43 \pm 0.10$, $r^2 = 0.51$) in *D. magna* and no significant relations in *G. roeseli*.

Size

Significant interspecies relations were found between accumulated Na, Fe, Mn, Cd, Co, and Zn and species weight (*W*) (see Fig. 3). Interspecies differences in bioaccumulation reflect allometric phenomena, caused by changes in surface to volume ratios.

Intraspecies relations were found for Mn, Fe, Co, and Cu. Intraspecies relations between C_b and *W* most likely reflect toxic effects of accumulated concentrations on growth instead of an allometric phenomenon as organisms were at the same life stage and size at the start of the experiment. Size variations of *D. magna* were related to accumulated concentrations of Fe (slope $= -1.35$, $r^2 = 0.32$), Co (slope $= -1.47$, $r^2 = 0.21$), and Cu (slope $= -0.59$, $r^2 = 0.33$), whereas *G. roeseli*'s size *s*howed a significant relation with accumulated Mn (slope $= -0.57$, $r^2 = 0.54$).

Multivariate bioaccumulation models

Significant multivariate models ($p < 0.05$) describing whole-body concentration as a function of exposure concentration, weight, or species could be derived for all metals in this study. From a pairwise *t* test with Holmes correction, it appeared that accumulation models containing f_{BL} (models 9–12) were significantly different from models without f_{BL} (models 1–8). The difference in AIC of 12 candidate regression models compared to the most simple reference model ($C_b =$ constant) is plotted in Figure 4.

Based on this comparison of AIC of candidate models, it appeared that whole-body concentrations of Ca, Na, and Zn were better described by a univariate model with either species ($C_b \sim$ Sp) or weight ($C_b \sim$ W) as variables. Fe, Mn, Cd, and Co whole-body concentrations were best described by a bivariate model: $C_b \sim C_w \times$ Sp. For Mg and Cu, none of the candidate models fulfilled this criterion; therefore, the reference model is selected as the best. Exposure concentrations, species, or weight did not contribute significantly to improved model performance for Mg, K, Ni, and Se. Models for C_b as a function of C_w and *W* as a function of C_w, *W*, and Sp as well as models with the highest statistical reliability are summarized in Table 4. Models for BCF are presented in the Supplemental Data, Table S2.

In general, species was selected as a better descriptor for bioaccumulation than weight. Because weight is an important species trait, some overlap in the predictive power of weight and species is to be expected. Univariate models for bioaccumulation as a function of weight are given in Figure 3. Multivariate models $C_b \sim C_w \times W$ performed less well than $C_b \sim C_w \times$ Sp, but still offered an acceptable *p* value and adjusted r^2 for Mn, Cd, and Co (see Table 4).

DISCUSSION

Regulation of whole-body metal concentrations

Strong regulatory systems to maintain optimal internal concentrations may partially explain the absence of significant relations between exposure concentrations and whole-body concentrations for Ca, Na, Mg, K, Fe, Cu, Se, and Zn. In *G. roeseli*, Cd is the only metal with a significant relation between C_w and C_b. In *D. magna*, besides Cd, two essential elements (Mn and Co) show significant relations with exposure concentrations, suggesting that regulatory systems are not able to maintain concentrations at an optimal level. Both Mn and Co also showed significant relations with f_{BL}, and the performance of f_{BL} as a model parameter was only slightly less than the performance of C_w. For *D. magna,* Ni accumulation (Fig. 2) also showed a trend ($b = 0.59$) with C_w, though this was nonsignificant due to the rather high variation in accumulated Ni concentrations.

A certain level of bioaccumulation is essential for normal physiological functions; that is, 84 μg Cu/g dry weight, 70 μg Zn/g dry weight, and 27 μg Fe/g dry weight were estimated to be the required amounts for optimal enzymatic functions in crustaceans [7,22]. Above certain concentrations in surface water, also essential metals will be accumulated, which is reflected by the functions for Mn and Co. From this perspective it can be concluded that Ca, Na, Mg, K, Fe, Cu, Se, and Zn concentrations at all the trial sites are within the physiological range, whereas Mn, Cd, Co, and Ni concentrations exceed physiological thresholds, resulting in increased whole-body concentrations.

Dissolved metal concentrations

Significant relations between exposure concentrations and BCFs of Cd, Cu, Ni, and Zn were found in the literature for a variety of species [1]. The absence of a significant relationship between C_b and C_w implies that internal body concentrations are constant ($b = 0$) and that BCF is inversely related to the exposure concentrations ($b' = -1$). Sometimes this distinction (either BCF is constant or C_b is constant) is not exclusive. Significant relations with exposure concentrations are found for C_b as well as for the corresponding BCF for Zn, Cd, Cu, and Ni

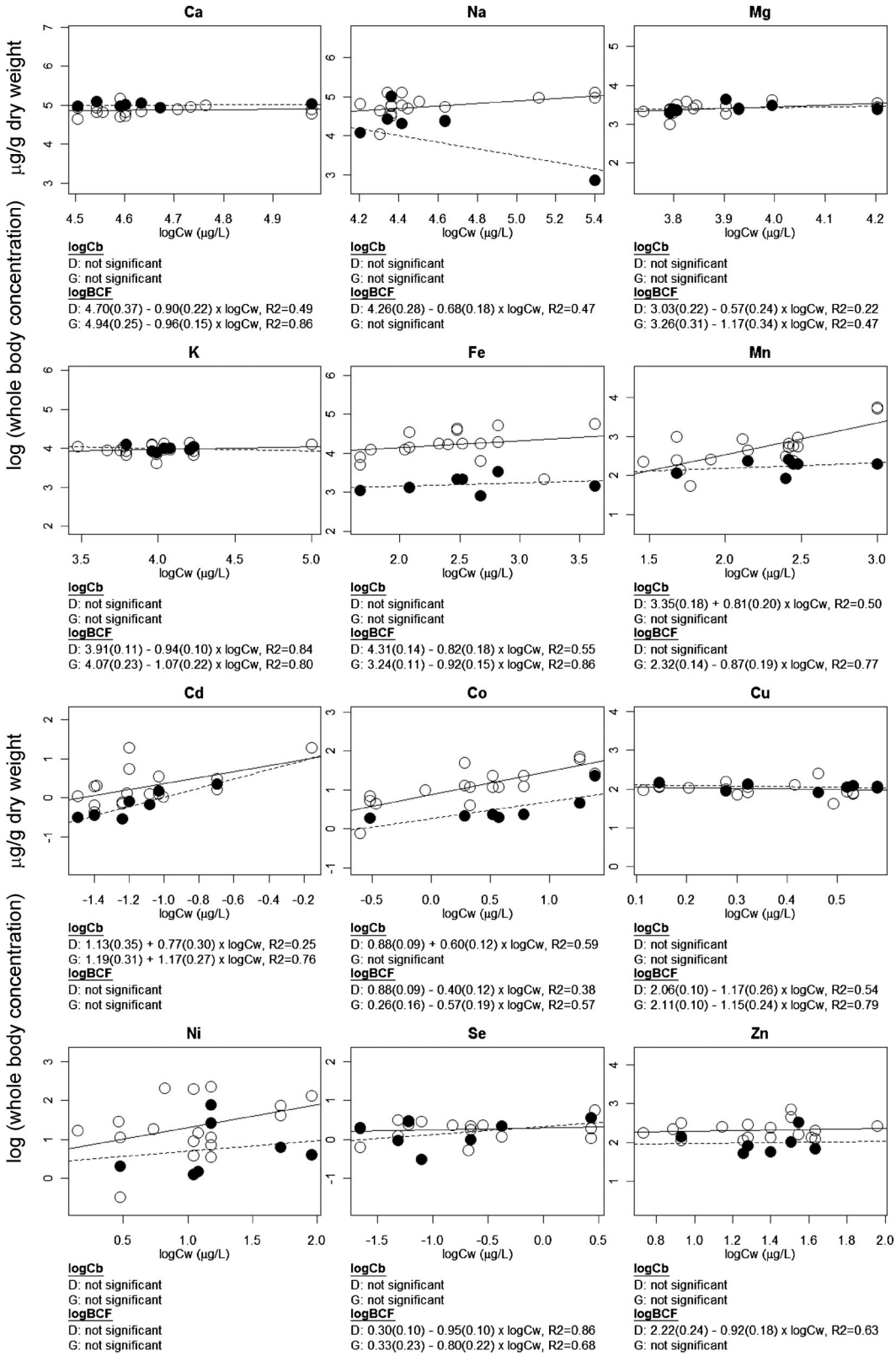

Fig. 2. Whole-body concentrations of metals in *Daphnia magna* (○, D) and *Gammarus roeseli* (●, G) related to dissolved metal concentrations (C_w) in 12 natural surface waters. Regression functions of $\log C_b \sim \log C_w$ and $\log BCF \sim \log C_w$ are printed below the figures, with standard error of the estimated coefficients in parentheses. Regressions are significant when slope is significantly different from zero ($p < 0.05$).

[1]. This is caused by the fact that accumulation levels off at higher exposure concentrations. Lebrun et al. [23] found that whole-body Ni concentrations proportionally increased with exposure concentrations in water up to 1,000 µg Ni/L. At higher Ni concentrations BCF leveled off. We observed a leveling off of Mn (b = 0.66) and Co (b = 0.55) accumulation.

In some cases neither C_b nor BCF was related to exposure (i.e., Ni in *D. magna*). This seems contradictory because one of

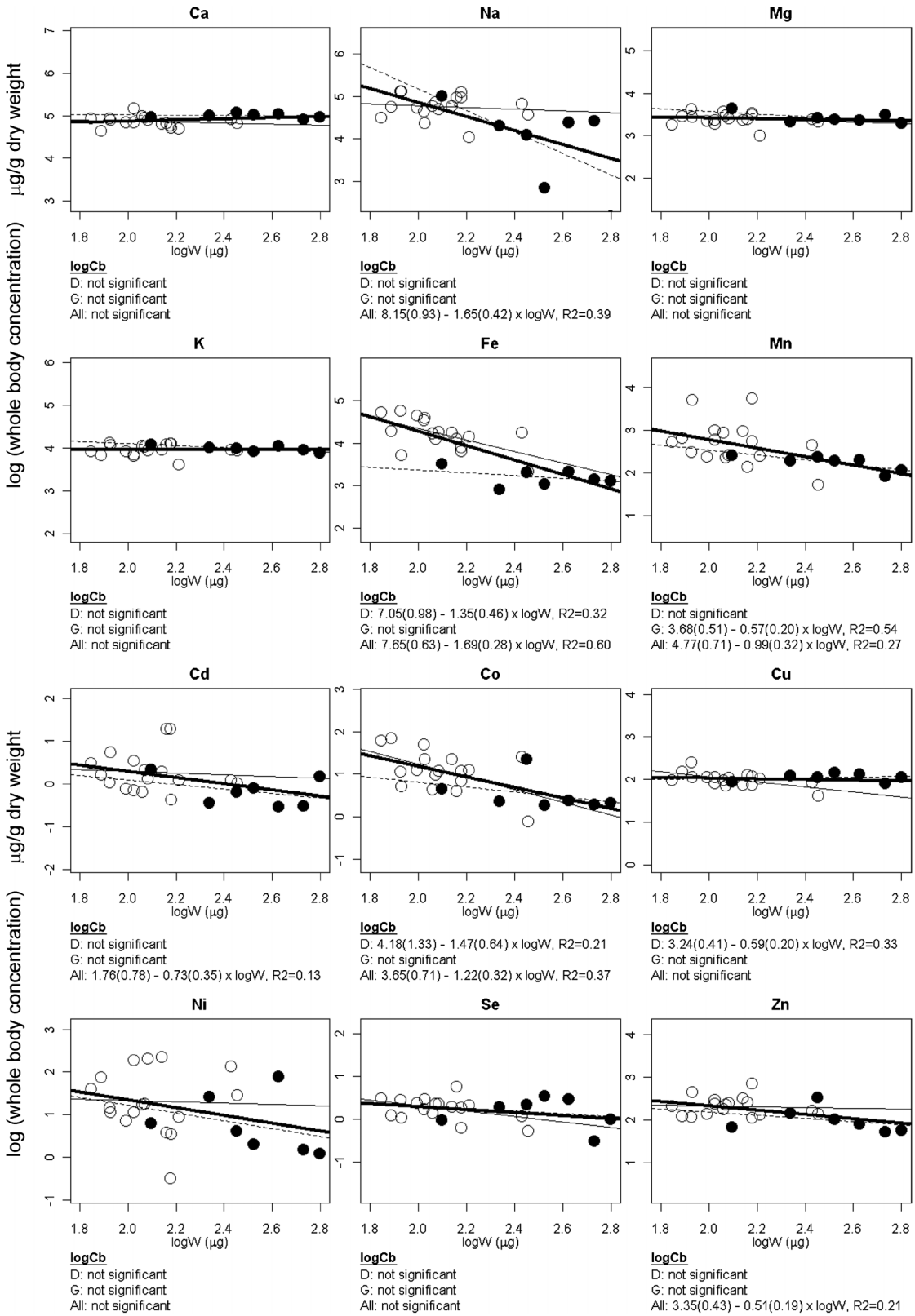

Fig. 3. Allometric relations between body weight (W, micrograms) and whole-body metal concentrations (C_b, micrograms per gram dry wt) in *Daphnia magna* (○, D) and *G. roeseli* (●, G) after three-week exposure in natural surface waters. Separate regression lines are shown for *D. magna* (—) and *G. roeseli* (). The bold line represents the regression over all data. Regressions are significant when the slope is significantly different from zero ($p < 0.05$). Significance, regressions, and slopes are similar for bioconcentration factors (BCFs). The BCF regression is not given because logBCF can easily be computed from regression of logC_b by logBCF = logC_b – logC_w.

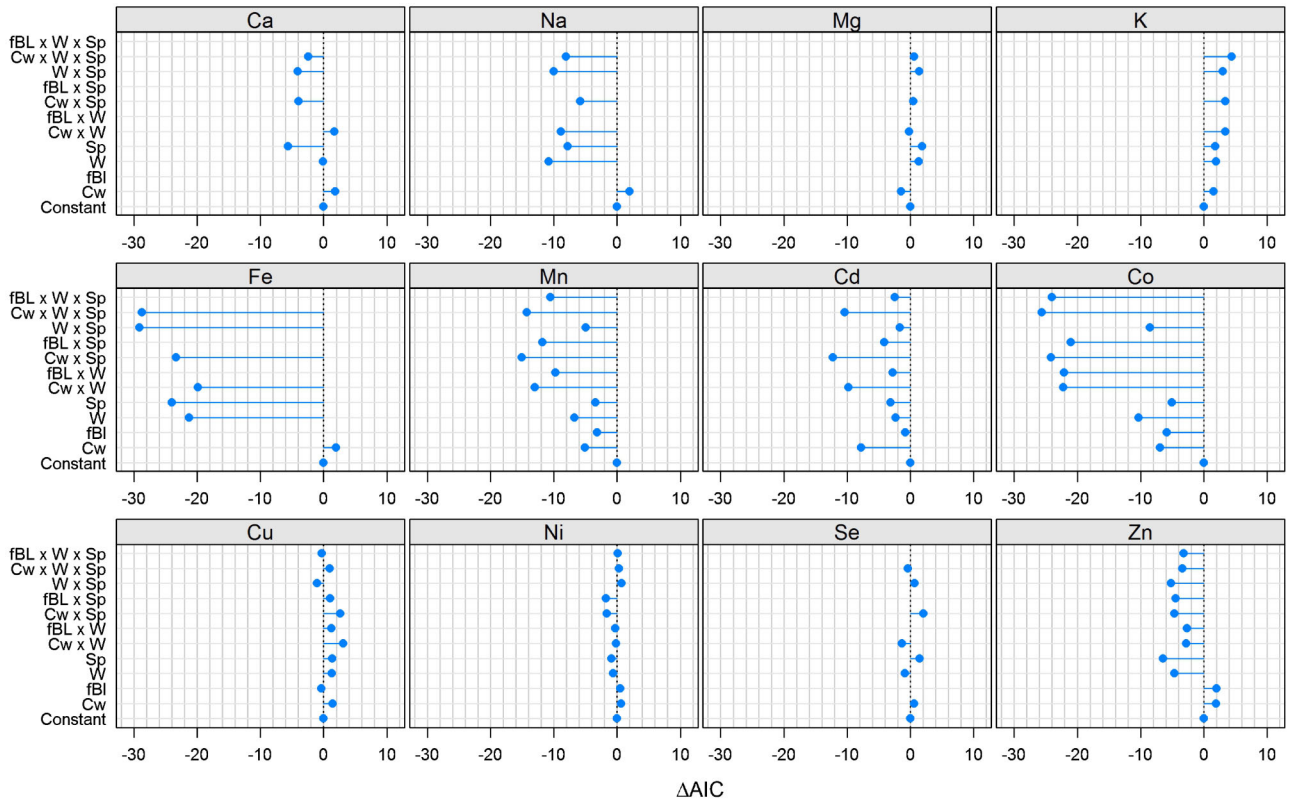

Fig. 4. Comparison of candidate models for prediction of whole-body concentrations of metals in *Daphnia magna* and *Gammarus roeseli*. ΔAIC = difference of Akaike information criterion between candidate models and the most simple reference model, $C_b \sim$ constant. Candidate models with ΔAIC <–2 are significantly better than the reference model. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com]

the options should be valid. For these cases, the statistical power of our data set is insufficient (caused by high variation coefficients of Ni–C_b) to conclude whether whole-body concentrations depend on aqueous concentration.

Absolute levels of BCF and whole-body concentrations are difficult to compare between studies because of the different expressions of concentrations in water (i.e., total concentrations [μg/L], dissolved concentrations [μg/L], standardized for suspended material [μg/kg], free metal ions [mol/L]) and in tissue (i.e., μg/kg wet wt or dry wt). Also, differences in analytical methods may introduce large differences between studies. The type and mesh of the filter used for dissolved metal sample preparation (very common are 0.22- and 0.45-μm filters) affect the measured concentration of dissolved metals. Preparation of tissue samples and analysis differ in many ways: depuration time for gut clearance, washing (EDTA) to remove metals adsorbed to cuticles, and destruction methods may introduce large differences between studies. Therefore, comparison between literature data and results in the present study is focused on the significance of allometric relations and not on the exact values of whole-body concentrations and BCF.

Occupancy of the biotic ligand

Exposure to metal mixtures affects the bioaccumulation of individual metals [24]. The relationship between exposure concentrations and whole-body concentrations might be masked by metal interactions, which are currently not very well understood. Norwood et al. [24] showed that Co, Cd, and Ni bioaccumulation in *Hyelella azteca* were significantly inhibited by increasing numbers of metals in the mixture, which included also As, Cr, Pb, Tl, Cu, Mn, and Zn. Bioaccumulation of Cu, Mn, and Zn was not significantly affected by exposure to other metals. Synergistic and antagonistic effects were also found for simultaneous uptake of Cd, Co, Ni, Pb, and Zn in *D. magna* and the zebrafish *Danio rerio*, which depended not only on the combination of metals but also on the exposure concentrations of the metals in the mixture [25,26].

In the present study, we found that biotic ligand binding did not improve the significance of the regression models of C_b and BCF compared with C_w. In combination with Sp, C_w performed significantly better than f_{BL} for Cd (ΔAIC = 8.10), Co (ΔAIC = 3.12), and Mn (ΔAIC = 3.28). Bioaccumulation of the other metals appeared to be independent of exposure concentrations (C_w or f_{BL}). The somewhat lower significance of the fractional occupancy of the biotic ligand for bioaccumulation prediction does not disqualify BLMs. Such models are suitable for effect assessment, often predicting effects within a factor of 2 [5,27,31]. Bioaccumulation of metals is not always directly related to effects because whole-body concentrations of essential metals are strongly regulated. Effects (i.e., reduced reproduction) are caused by energy costs involved in maintaining body concentrations at a constant level. Therefore, effects, rather than bioaccumulation, are expected to be related to metal concentrations in the exposure medium or to the amount of metal bound to gill or gill-like organs. Moreover, whereas C_w is a measured value, f_{BL} is an estimated value, which contains uncertainties caused by the calculation of free ion activities and the use of a conditional affinity constant for binding to the biotic ligand.

Biological variation

Increasing whole-body concentrations are found at lower exposure concentrations in *D. magna* than in *G. roeseli.* This might be caused by different boundaries of its regulatory

Table 4. Bivariate, trivariate, and best models for C_b (micrograms per gram dry wt) as a function of dissolved metal concentration (C_w, micrograms per liter), species weight (W, micrograms per gram dry wt), and other undefined species traits (Sp)

		AIC	p	Adj. r^2
Model: $\log C_b = a\log C_w + \kappa\log W$				
Ca	$4.49(0.37) + 0.07(0.18)\log C_w + 0.14(0.10)\log W$	−97.0	0.365	0.01
Na	$8.31(1.10) - 0.09(0.31)\log C_w - 1.66(0.43)\log W$	−25.8	0.004	0.36
Mg	$3.27(0.28) + 0.35(0.19)\log C_w - 0.08(0.10)\log W$	−96.5	0.160	0.08
K	$3.99(0.22) + 0.06(0.09)\log C_w - 0.04(0.09)\log W$	−98.5	0.765	−0.07
Fe	$7.74(0.64) + 0.10(0.14)\log C_w - 1.7(0.29)\log W$	−45.3	<0.0001	0.59
Mn	$4.95(0.62) + 0.50(0.17)\log C_w - 0.91(0.28)\log W$	−47.0	5.8e-4	0.46
Cd	$2.34(0.68) + 0.81(0.26)\log C_w - 0.57(0.3)\log W$	−44.0	0.002	0.38
Co	$3.31(0.55) + 0.42(0.1)\log C_w - 1.16(0.25)\log W$	−52.4	<0.0001	0.63
Cu	$2.21(0.26) - 0.10(0.21)\log C_w - 0.07(0.12)\log W$	−88.5	0.664	−0.05
Ni	$2.76(1.31) + 0.36(0.31)\log C_w - 0.9(0.56)\log W$	−12.4	0.164	0.08
Se	$1.24(0.50) + 0.12(0.09)\log C_w - 0.41(0.22)\log W$	−56.2	0.112	0.12
Zn	$3.28(0.49) + 0.06(0.18)\log C_w - 0.51(0.20)\log W$	−63.0	0.051	0.18
Model: $\log C_b = a\log C_w + \kappa\log W + \lambda Sp$				
Ca	$4.89(0.38) + 0.09(0.16)\log C_w - 0.08(0.13)\log W + 0.18(0.07)Sp$	−101.1	0.066	0.19
Na	$7.42(1.41) - 0.08(0.31)\log C_w - 1.21(0.61)\log W - 0.36(0.35)Sp$	−25.0	0.007	0.36
Mg	$3.51(0.37) + 0.31(0.20)\log C_w - 0.18(0.14)\log W + 0.08(0.08)Sp$	−95.7	0.202	0.08
K	$4.16(0.29) + 0.07(0.09)\log C_w - 0.12(0.14)\log W + 0.07(0.08)Sp$	−97.5	0.715	−0.08
Fe	$6.16(0.70) + 0.14(0.12)\log C_w - 0.89(0.33)\log W - 0.66(0.19)Sp$	−54.1	<0.0001	0.73
Mn	$4.02(0.80) + 0.61(0.17)\log C_w - 0.40(0.40)\log W - 0.40(0.23)Sp$	−48.3	6.2e-4	0.51
Cd	$1.48(0.87) + 0.83(0.25)\log C_w - 0.13(0.41)\log W - 0.36(0.24)Sp$	−44.6	0.003	0.42
Co	$2.16(0.72) + 0.50(0.10)\log C_w - 0.59(0.34)\log W - 0.45(0.20)Sp$	−55.9	<0.0001	0.69
Cu	$2.65(0.34) - 0.02(0.20)\log C_w - 0.31(0.17)\log W + 0.18(0.09)Sp$	−90.5	0.232	0.07
Ni	$1.24(1.84) + 0.48(0.33)\log C_w - 0.20(0.82)\log W$ - $0.57(0.49)Sp$	−12.0	0.181	0.09
Se	$1.68(0.69) + 0.14(0.09)\log C_w - 0.63(0.32)\log W + 0.17(0.18)Sp$	−55.2	0.161	0.11
Zn	$2.68(0.62) + 0.07(0.17)\log C_w - 0.22(0.27)\log W - 0.24(0.16)Sp$	−63.6	0.046	0.22
Best model: log C_b =				
Ca	$4.87\ (0.03) + 0.14\ (0.05)\ Sp$	−104.8	<0.0001	0.23
Na	$8.15\ (0.93) - 1.65\ (0.42)\ \log W$	−27.7	7.0e-4	0.39
Mg	3.42 (0.03)	−96.3	<0.0001	
K	3.98 (0.02)	−101.9	<0.0001	
Fe	$6.10\ (0.71) - 0.90\ (0.34)\ \log W - 0.64\ (0.20)\ Sp$	−54.5	<0.0001	0.72
Mn	$3.23\ (0.15) + 0.66\ (0.16)\ \log C_w - 0.57\ (0.15)\ Sp$	−49.1	2.4e-4	0.51
Cd	$1.22\ (0.28) + 0.84\ (0.24)\ \log C_w - 0.41\ (0.16)\ Sp$	−46.4	8.2e-4	0.44
Co	$0.90\ (0.08) + 0.55\ (0.10)\ \log C_w - 0.71\ (0.14)\ Sp$	−54.4	<0.0001	0.66
Cu	2.02 (0.03)	−82.7	<0.0001	
Ni	1.15 (0.15)	−13.1	<0.0001	
Se	0.23 (0.06)	−61.0	<0.0001	
Zn	$2.32\ (0.06) - 0.33\ (0.11)\ Sp$	−70.9	5.9e-3	0.27

Sp = 0 for *D. magna*; Sp = 1 for *G. roeseli*.

system. Another explanation might be that *D. magna* feeds on naturally occurring algae, whereas *G. roeseli* feeds on leaves added into the cages. We assume that algal quality reflects water quality, whereas the quality of the leaves is independent of the water chemistry at the trial sites. Relationships between water quality and whole-body concentrations are stronger in the filter feeder *D. magna* because whole-body concentrations are built up from waterborne as well as dietary exposure, whereas concentrations in the shredder *G. roeseli* originate from waterborne exposure only. The regression analysis shows that interspecies variation is important to take into account and, for some metals, is the only significant parameter.

Because weight is an important species trait, overlap in the predictive power of weight and species as a covariable is to be expected. The coefficient for species as a covariable is a lumped parameter that contains all undefined species traits that result in different bioaccumulation. Besides size, other traits affect bioaccumulation such as filtration rate, food consumption, uptake rate, elimination rate, storage capacity, detoxification. The present study shows that it is essential to account for intrinsic differences in bioaccumulation between species.

Size

Significant intraspecies relations between body weight and accumulated metals were found for Fe, Mn, Cu, and Co in adult *D. magna* and for Mn in *G. roeseli*. Size variations of *D. magna* adults could be explained by accumulated concentrations of Fe, Co, and Cu, whereas *G. roeseli*'s size showed a significant relation with accumulated Mn. The relation between accumulated Co and weight of *D. magna* adults is not unexpected, as the surface waters in the present study cover a broad concentration range from low values to values far above the environmental quality standard. The effects of Cu, Fe, and Mn are more difficult to understand because aqueous concentrations do not indicate a risk for these elements. Elevated whole-body concentrations of Cu, Fe, and Mn can be associated with the respiratory system. Copper is a constituent of hemocyanin, which is involved in oxygen binding. The level of hemocyanin in organisms is related to the oxygen tension in the environment. Elevated manganese concentrations were observed in the marine benthic crustacean *Nephrops norvegicus* proportional to the duration of the hypoxic event and the exposure concentrations [29]. Although in general the aeration in the streams in our study is good, temporary fluctuations or temporary hypoxia during the experiment cannot be excluded.

Statistical power

The significance of effects is more difficult to prove in field tests than in laboratory tests, due to the inherently higher variability caused by variation of environmental conditions

such as water temperature, aeration, and availability and quality of food. Moreover, exposure concentrations are not controlled and vary in time. Variation in body weight can be caused by the effect of the exposure medium on development, growth, and reproduction. Molting and release of offspring cause variation in body weight of *D. magna*. Up to 70% of Cd and Zn uptake in *D. magna* can be lost by molting, and up to 70% of Se uptake can be removed by the release of offspring [30]. In our experiment *G. roeseli* did not produce offspring. Variation in the weight of *G. roeseli* can be caused by sex differences and small age differences. At the start of the experiment, *G. roeseli* were 1 to 7 d old and sex differences were not visible.

Less significant relations were found for *G. roeseli* than for adult *D. magna* because data for these organisms are less abundant in our data set (see Table 2). Data on *G. roeseli* at eight out of 12 sites were obtained. The high variation and smaller number of trial sites resulted in a data set with less statistical power for *G. roeseli*.

CONCLUSION

Differences in bioaccumulation between species were observed for most of the elements in this study and often related to differences in weight. As far as effects are concerned, Co, Cu, and Fe accumulation coincided with reduced growth of *D. magna* and Mn was related to reduced growth of *G. roeseli*. Smaller organisms accumulated higher amounts of metals per gram body weight compared to larger organisms for Na, Fe, Mn, Cd, Co, and Zn, following an allometric model.

Field-exposed organisms accumulated increasing amounts of Mn, Cd, Co, and Ni with increasing dissolved metal concentrations; but accumulation gradually stabilized at higher concentration levels. Significant relations were found between bioaccumulation of Mn and Co and both fractional occupancy of the biotic ligand and dissolved metal concentrations, where the latter proved to be more significant.

SUPPLEMENTAL DATA

Table S1. Calculated free ion activities of tested surface waters.

Table S2. Bivariate, trivariate, and best models for BCF as a function of dissolved metal concentration, species weight, and other undefined species traits.

Figure S1. Comparison of candidate models for prediction of BCF of metals in *D. magna* and *G. roeseli*. (2 MB DOC)

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