

MODELING METAL BIOACCUMULATION IN THE INVASIVE MUSSELS  
*DREISSENA POLYMORPHA* AND *DREISSENA ROSTRIFORMIS BUGENSIS*  
IN THE RIVERS RHINE AND MEUSE

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(Submitted 21 June 2011; Returned for Revision 18 July 2011; Accepted 29 August 2011)

**Abstract**—The metal-specific covalent index and the species-specific size-based filtration rate were integrated into a biokinetic model estimating metal bioaccumulation in mussels from the dissolved phase and phytoplankton. The model was validated for zebra (*Dreissena polymorpha*) and quagga (*Dreissena rostriformis bugensis*) mussels in the rivers Rhine and Meuse, the Netherlands. The model performed well in predicting tissue concentrations in different-sized zebra mussels from various sampling sites for  $^{55}\text{Mn}$ ,  $^{56}\text{Fe}$ ,  $^{59}\text{Co}$ ,  $^{60}\text{Ni}$ ,  $^{82}\text{Se}$ ,  $^{111}\text{Cd}$ ,  $^{113}\text{Sn}$ , and  $^{208}\text{Pb}$  ( $r^2 = 0.71\text{--}0.99$ ). Performance for  $^{52}\text{Cr}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{68}\text{Zn}$ , and  $^{112}\text{Cd}$  was moderate ( $r^2 < 0.20$ ). In quagga mussels, approximately 73 to 94% of the variability in concentrations of  $^{82}\text{Se}$ ,  $^{111}\text{Cd}$ ,  $^{112}\text{Cd}$ , and  $^{208}\text{Pb}$  was explained by the model ( $r^2 = 0.73\text{--}0.94$ ), followed by  $^{52}\text{Cr}$ ,  $^{55}\text{Mn}$ ,  $^{56}\text{Fe}$ ,  $^{60}\text{Ni}$ , and  $^{63}\text{Cu}$  ( $r^2 = 0.48\text{--}0.61$ ). Additionally, in both zebra and quagga mussels, average modeled concentrations were within approximately one order of magnitude of the measured values. In particular, in zebra mussels, estimations of  $^{60}\text{Ni}$  and  $^{82}\text{Se}$  concentrations were equal to 51 and 76% of the measurements, respectively. Higher deviations were observed for  $^{52}\text{Cr}$ ,  $^{59}\text{Co}$ ,  $^{55}\text{Mn}$ ,  $^{56}\text{Fe}$ ,  $^{111}\text{Cd}$ ,  $^{63}\text{Cu}$ , and  $^{112}\text{Cd}$  (underestimation), and  $^{66}\text{Zn}$ ,  $^{68}\text{Zn}$ ,  $^{208}\text{Pb}$ , and  $^{113}\text{Sn}$  (overestimation). For quagga mussels, modeled concentrations of  $^{66}\text{Zn}$  and  $^{68}\text{Zn}$  differed approximately 14% from the measured levels. Differences between predictions and measurements were higher for other metals. Environ. Toxicol. Chem. 2011;30:2825–2830. © 2011 SETAC

**Keywords**—Bivalve    Modeling    Bioconcentration factor    River Rhine    River Meuse

## INTRODUCTION

Because of its widespread distribution and high efficiency in filtering particulate matter, the zebra mussel (*Dreissena polymorpha*) has been used in numerous monitoring programs [1,2]. Biomonitoring studies showed high metal concentrations in mussels from the rivers Rhine and Meuse [1]. Recently, attention has been drawn to the displacement of the zebra mussel by the quagga mussel (*Dreissena rostriformis bugensis*) [3]. Quagga mussels are more tolerant to unfavorable conditions, such as bad water quality or low availability of food, and metal pollution [4]. Understanding metal bioaccumulation in these two species may provide insight into differences in tolerance to metal exposure. This may be one of the driving forces for the ongoing changes in the population structure of dreissenid mussels. This information is also useful in assessing potential effects on their predators, such as benthivorous fish and diving ducks.

With recognized difficulties in assessing metal bioaccumulation and toxicity, kinetic-based models have been recommended as a potential method for predicting metal bioaccumulation [5]. The models also enable distinguishing accumulation from dissolved and dietary sources. This distinction is important because exposure type determines internal distribution and eventually toxicity [6]. In mechanistic bioaccumulation models for organic chemicals, absorption, assimilation, and elimination rate constants are usually related to substance-specific properties, such as the octanol–water partition coefficient, and physiological features such as size [7]. The

advantage of these models lies in the potential for extrapolation to a wide range of pollutants, species, and conditions without case-specific calibration [8]. Yet, similar models for metals are rare because of the complex chemistry of metals in the environment [8–10]. So far, parameters in metal models, that is, the physiological rate constants, should be experimentally measured on a case-by-case basis. Relating these constants to metal-specific properties increases extrapolation potential for a number of metals. Recent studies indicate possibilities of integrating the metal-specific covalent index into modeling metal bioaccumulation. In particular, significant relationships were found between the covalent index and metal bioaccumulation in mollusks [11]. In addition, variability in metal absorption rate constants between mussels and other species was shown to be primarily a function of the filtration rate [8]. The integration of the species-specific filtration rate as well as other physiological processes may provide a better understanding of the differences in the sensitivities of the zebra and quagga mussels to metal exposure.

In the present study, we aimed to integrate the covalent index and the size-based filtration rate into a kinetic model simulating metal bioaccumulation in zebra and quagga mussels in the rivers Rhine and Meuse. The developed model was then validated by using data from field measurements in these rivers.

## METHODS

*Specification and parameterization of the model*

Trace metals can be accumulated in mussels from the dissolved phase and particulate matters or phytoplankton [12–14]. In the present study, phytoplankton was considered the main source of food for mussels. Metal concentrations in mussels  $C_m$  ( $\mu\text{g/g}$  dry wt) were regarded to be determined by uptake via water (the first factor) and food (the second factor)

All Supplemental Data may be found in the online version of this article.

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Published online 23 September 2011 in Wiley Online Library  
(wileyonlinelibrary.com).

and by losses through elimination and growth dilution (the last factor) (Eqn. 1). These factors are specified later.

$$\frac{dC_m}{dt} = (p \times FR \times C_w) + (IR \times AE \times C_f) - (k_{ew} + k_{ef} + g) \times C_m \quad (1)$$

where  $p$  is the absorption efficiency,  $FR$  is the filtration rate,  $C_w$  is the dissolved metal concentration,  $IR$  is the ingestion rate,  $AE$  is the assimilation efficiency,  $C_f$  is the metal concentration in food,  $k_{ew}$  is the elimination rate via water,  $k_{ef}$  is the elimination rate via food, and  $g$  is the growth rate.

**Uptake of metals from the dissolved phase.** Metal uptake from water is a function of the dissolved metal concentration  $C_w$  ( $\mu\text{g/L}$ ) and the absorption rate  $k_u$  ( $\text{L/g dry wt/d}$ ). The absorption rate was considered metal- and species-specific [6]. It depends on biological factors, such as the filtration rate  $FR$  ( $\text{L/g dry wt/d}$ ), and the metal-specific absorption efficiency  $p$  (%) [15,16].

At low food levels, the filtration rate is generally independent of food concentration [17]. After reaching certain levels, the rate will decrease as a function of food availability [17,18]. Therefore, the filtration rate was considered to be a function of food concentration  $F$  (in phytoplankton biomass,  $\text{g/L}$ ), the maximum filtration rate  $FR_{\text{max}}$  ( $\text{L/g dry wt/d}$ ), and the saturation constant  $K_m$  ( $\text{g/L}$ ) (Eqn. 2). The saturation constant  $0.04 \text{ g/L}$  was derived from the value of  $20 \text{ mg C/L}$  determined by Descy et al. [19] by using the conversion factor of 2 between organic carbon content and phytoplankton biomass suggested by Roditi et al. [20]. The filtration rate is mainly measured per individual (e.g.,  $\text{L/mussel/h}$ ) and depends on the mussel size, or dry weight, according to the power function  $a \cdot W^b$  with various values of  $a$  and  $b$  reported [21,22]. For zebra mussels, the maximum individual-based filtration rate  $FR_m$  ( $\text{L/mussel/h}$ ) and dry weight  $W$  ( $\text{g}$ ) were taken from the widely applied relationships found by Kryger and Riisgard [21] (Eqns. 3–4). The filtration rate found by Kryger and Riisgard [21] was high because of the optimal experimental conditions [23] and therefore considered maximum in the present study. For quagga mussels, the allometric equations from the study by Baldwin et al. [24] were used to determine the  $FR_m$  ( $\text{L/mussel/h}$ ) and dry weight  $W$  ( $\text{g}$ ) (Eqns. 5–6). These individual-based filtration rates were transferred into the mass-specific form  $FR_{\text{max}}$  ( $\text{L/g dry wt/d}$ ) applied in the model. The pseudo feces production, which may act as a mechanism to clear excess particles or to reject some particle types, was excluded in the current model [18] (see Discussion section).

$$FR = \frac{FR_{\text{max}} \times K_m}{K_m + F} \quad (2)$$

$$FR_m = 6.82 \times W^{0.88} \quad (3)$$

$$W = 1.54 \times 10^{-5} \times SL^{2.42} \quad (4)$$

$$FR_m = 0.7866 \times W^{0.6266} \quad (5)$$

$$W = 0.0209 \times SL^{2.53} \quad (6)$$

According to Wang and Fisher [25], the metal absorption efficiency is independent of the filtration rate among mussels in different size classes. Available data on this efficiency for mussels, especially the quagga mussel, are limited. We therefore used the relationship between the metal absorption efficiency  $p$  and the covalent index  $X_m^2 r$  developed by Veltman

et al. [8] (Eqn. 7).

$$\log \left[ \frac{p}{1-p} \right] = 0.57 \times [X_m^2 r] - 4.37 \quad (7)$$

**Uptake of metals from food.** Metal uptake from food by mussels is a function of the ingestion rate  $IR$  ( $\text{g/g dry wt/d}$ ), the metal assimilation efficiency ( $AE$ , %), and the metal concentration in ingested food  $C_f$  ( $\mu\text{g/g}$ ) [16]. All particles filtered by mussels were assumed to be ingested with greater than 90% retention efficiencies for different-typed particles [20,22,26]. The ingestion rate ( $\text{g/g dry wt/d}$ ) therefore equaled the amount of food ( $\text{g/L}$ ) contained in the filtered water ( $\text{L/g dry wt/d}$ ) (Eqn. 8).

$$IR = FR \times F \quad (8)$$

The assimilation efficiency is the percentage of ingested metals crossing gut lining. No statistically significant relationship was found between this parameter and the covalent index based on data from the study by Roditi et al. [14]. Therefore, available data collected from previous studies were applied directly to the model for Cd, Cr, Se, Co, Zn, and Pb (Supplemental Data, Table S2).

Metal concentrations in phytoplankton  $C_f$  ( $\mu\text{g/g}$ ) were calculated from bioconcentration factors ( $BCF$ ) of phytoplankton  $BCF_p$  ( $\text{L/kg}$ ) and dissolved metal concentrations  $C_w$  ( $\text{mg/L}$ ) (Eqn. 9). The  $BCF_p$  is metal-specific and dependent on exposure concentrations [27]. However, the dependence on exposure concentrations was not included in modeling  $BCF_p$  to simplify the extrapolation for particular environmental conditions. To reduce uncertainties from this simplification,  $BCF_p$  data were included only if the dissolved metal concentrations did not differ considerably from measurements in the rivers Rhine and Meuse (Supplemental Data, Table S3). Collected  $BCF_p$  values were found not to be significantly correlated to the covalent index and, consequently, directly applied to the model.

$$C_f = BCF_p \times C_w \quad (9)$$

**Elimination.** Metals may be lost via water and food with elimination rates  $k_{ew}$  and  $k_{ef}$  ( $/\text{d}$ ), respectively [14,15]. Elimination rates are inversely proportional to species weight by a factor of  $(-0.25)$  [27]. Weight-corrected elimination rates are metal-specific, but studies relating these rates to the mussel size are limited [8]. Therefore, in the present study, elimination rates reported by Roditi et al. [14] were considered weight-corrected for mussels with the standardized dry weight. This standardized dry weight was assumed to correspond to 20-mm shell length. The weight-corrected elimination rates were related to the covalent index (Supplemental Data, Table S4 and Fig. S1). The elimination rates were therefore expressed as follows:

$$k_{ew} = 10^{-1.93} \times [X_m^2 r]^{0.91} \times \left( \frac{W}{W_s} \right)^{-0.25} \quad (10)$$

$$k_{ef} = 10^{-2.24} \times [X_m^2 r]^{1.44} \times \left( \frac{W}{W_s} \right)^{-0.25} \quad (11)$$

where  $W_s$  denotes the standardized dry weight.

Tissue concentrations also may decrease by growth dilution, which is proportional to the growth rate  $g$  ( $/\text{d}$ ). The growth rate depends on, for example, initial size, temperature, and food availability [28,29]. At the relatively constant environmental conditions, it was assumed that the initial mussel size is the

decisive factor, determining the growth rate. For zebra mussels, the relationship between the shell length added per day (SLA) and the initial shell length (SL) (mm) developed by Stoeckmann and Garton [30] was used because of the wide range of size classes studied (Eqn. 12). The mass-based growth rate applied in the model was derived from this increase in shell length by using Equation 4 relating dry weight to shell length. According to Baldwin et al. [24], the growth rate of quagga mussels is 4 to 19 times higher than that of zebra mussels. The difference between their growth rates increases with a decrease in food availability. The chlorophyll *a* concentrations measured in the rivers Rhine and Meuse were similar to the highest levels studied by Baldwin et al. As a result, a fourfold higher growth rate compared with that of the zebra mussel was assumed for the quagga mussel.

$$\text{SLA} = 0.0795 - 0.00347 \times \text{SL} \quad (12)$$

### Sampling and chemical analysis

**Mussel and water samples.** Zebra and quagga mussels were collected from groynes at Lexkesveer in the River Rhine and at Middelaar in the River Meuse in April 2010. These sites were selected based on available evidence on the co-existence of the two species. At each site, river water samples were taken and filtered using a Whatman GFC Glass Microfiber Filter of 47 mm (cat. 1822-047). Filters were then dried for 24 h at 60°C to determine the dry weight of suspended solids.

**Sample preparation and analysis.** In total, 424 and 688 individual mussels collected from the rivers Rhine and Meuse, respectively, were grouped into three size classes: small (<15 mm), medium (15–22 mm), and large (≥22 mm). The fresh parts were separated and dried at 60°C for 48 h. The dried fresh fraction was then digested with a mixture of HNO<sub>3</sub> 65% and H<sub>2</sub>O<sub>2</sub> in the Milestone Ethos-D microwave. For each sample, 0.2 g of the dried fresh weight was digested by 4 ml of HNO<sub>3</sub> and 0.5 ml of H<sub>2</sub>O<sub>2</sub>. Small- and medium-sized mussels showed higher abundance than larger ones. Therefore, for small- and medium-sized classes, two or three samples were measured to increase the representativeness of the examined samples. The cooled digests were made up to exactly 100 ml with high-quality deionized water. A similar procedure was performed for blank samples for corrections to determine metal concentrations in experimental samples. Diluted digests and filtered water samples were analyzed for <sup>52</sup>Cr, <sup>55</sup>Mn, <sup>56</sup>Fe, <sup>59</sup>Co, <sup>60</sup>Ni, <sup>63</sup>Cu, <sup>66</sup>Zn, <sup>68</sup>Zn, <sup>82</sup>Se, <sup>111</sup>Cd, <sup>112</sup>Cd, <sup>118</sup>Sn, and <sup>208</sup>Pb by inductively coupled plasma-mass spectroscopy.

### Model validation

The model was validated by combining the measurement data in the present study and monitoring data from the Netherlands Monitoring Waterstaatkundige Toestand des Lands. Monitoring data measured at upstream monitoring stations Lobith and Belfeld in 2008 and 2009 were used for the sampling sites at Lexkesveer and Middelaar, respectively. Particularly, the measurements of suspended solids together with monitoring data about percentage of organic carbon in suspended matters (Supplemental Data, Table S5) were used to calculate concentrations of organic carbon. Phytoplankton biomass was then derived from the organic carbon content using the conversion factor of 2 as described previously. Together with measurements of mussel shell length (Supplemental Data, Table S6) and dissolved metal concentrations (Supplemental Data, Table S7), the data on the phytoplankton biomass was used to determine modeled metal

concentrations in mussel tissues. These results were compared with measured values for corresponding size classes (Supplemental Data, Table S8). The explanatory power of the model was assessed by the coefficient of determination (*r*<sup>2</sup>) between modeled and measured tissue concentrations for each metal. Data on BCF<sub>p</sub> and assimilation efficiency were available for <sup>52</sup>Cr, <sup>59</sup>Co, <sup>66</sup>Zn, <sup>68</sup>Zn, <sup>82</sup>Se, <sup>111</sup>Cd, <sup>112</sup>Cd, and <sup>208</sup>Pb, so both the dissolved phase and food were taken into validation for these metals. For <sup>55</sup>Mn, <sup>56</sup>Fe, <sup>60</sup>Ni, <sup>63</sup>Cu, and <sup>118</sup>Sn, only water was included in model validation, because no available or derived data of these parameters were found. The relative contribution from food *U<sub>f</sub>* (%) was calculated as the percentage of the uptake from food in total uptake (Eqn. 13).

$$U_f = \frac{\text{IR} \times \text{AE} \times C_f}{p \times \text{FR} \times C_w + \text{IR} \times \text{AE} \times C_f} \times 100\% \quad (13)$$

## RESULTS

### Validation results

Approximately 71 to 99% of the variability in tissue concentrations of <sup>55</sup>Mn, <sup>56</sup>Fe, <sup>59</sup>Co, <sup>60</sup>Ni, <sup>82</sup>Se, <sup>111</sup>Cd, <sup>118</sup>Sn, and <sup>208</sup>Pb in zebra mussels at different sampling sites and in various size classes was explained by the model (*r*<sup>2</sup> = 0.71–0.99; Supplemental Data, Fig. S2; Table S9). The explained variance in tissue concentrations was lower for <sup>52</sup>Cr, <sup>63</sup>Cu, <sup>66</sup>Zn, <sup>68</sup>Zn, and <sup>112</sup>Cd (*r*<sup>2</sup> < 0.20). For different-sized quagga mussels taken from various sites, the best performance of the model was noted for <sup>82</sup>Se, <sup>111</sup>Cd, <sup>112</sup>Cd, and <sup>208</sup>Pb (*r*<sup>2</sup> = 0.73–0.94), followed by <sup>52</sup>Cr, <sup>55</sup>Mn, <sup>56</sup>Fe, <sup>60</sup>Ni, and <sup>63</sup>Cu (*r*<sup>2</sup> = 0.48–0.61; Supplemental Data, Fig. S3; Table S9). In contrast, only 4 to 25% of the variability in tissue concentrations of <sup>59</sup>Co, <sup>66</sup>Zn, <sup>68</sup>Zn, and <sup>118</sup>Sn was explained by the model.

In general, average modeled concentrations for both the zebra and quagga mussels were below measured levels, except for <sup>66</sup>Zn, <sup>68</sup>Zn, <sup>208</sup>Pb, and <sup>118</sup>Sn, by approximately one order of magnitude (Fig. 1). In zebra mussels, estimations for <sup>60</sup>Ni and <sup>82</sup>Se concentrations agreed most, equaling 51 and 76% of the field measurements, respectively (Fig. 1a). Deviations between predictions and measurements were higher for other metals. Concentrations of <sup>52</sup>Cr, <sup>59</sup>Co, <sup>55</sup>Mn, <sup>56</sup>Fe, <sup>111</sup>Cd, <sup>63</sup>Cu, and <sup>112</sup>Cd were underestimated, whereas those of <sup>66</sup>Zn, <sup>68</sup>Zn, <sup>208</sup>Pb, and <sup>118</sup>Sn were overestimated. For quagga mussels, modeled concentrations of <sup>66</sup>Zn and <sup>68</sup>Zn differed from the measured levels by approximately 14% (Fig. 1b). Higher differences were found between predictions and measurements for other metals.

### Metal bioaccumulation in mussels

In both rivers Rhine and Meuse, highly significant relationships were found between metal concentrations in zebra mussels and in quagga mussels (*p* < 0.0001; Table 1). Metal concentrations in the mussels in the river Rhine were significantly lower than those in the river Meuse (*p* < 0.0001). In addition, the relative contribution from the two uptake pathways was metal-specific, with a dominant fraction from food for <sup>66</sup>Zn and <sup>68</sup>Zn and from water for <sup>52</sup>Cr, <sup>82</sup>Se, <sup>111</sup>Cd, <sup>112</sup>Cd, and <sup>208</sup>Pb (Supplemental Data, Table S10).

Bioconcentration factors and bioaccumulation factors were calculated as the ratio between metal concentrations in organisms, without and with uptake from food, respectively, versus metal concentrations in water. In both species, significant relationships were found between the BCF and bioaccumulation factors values (*p* < 0.0001; Supplemental Data, Table S10).

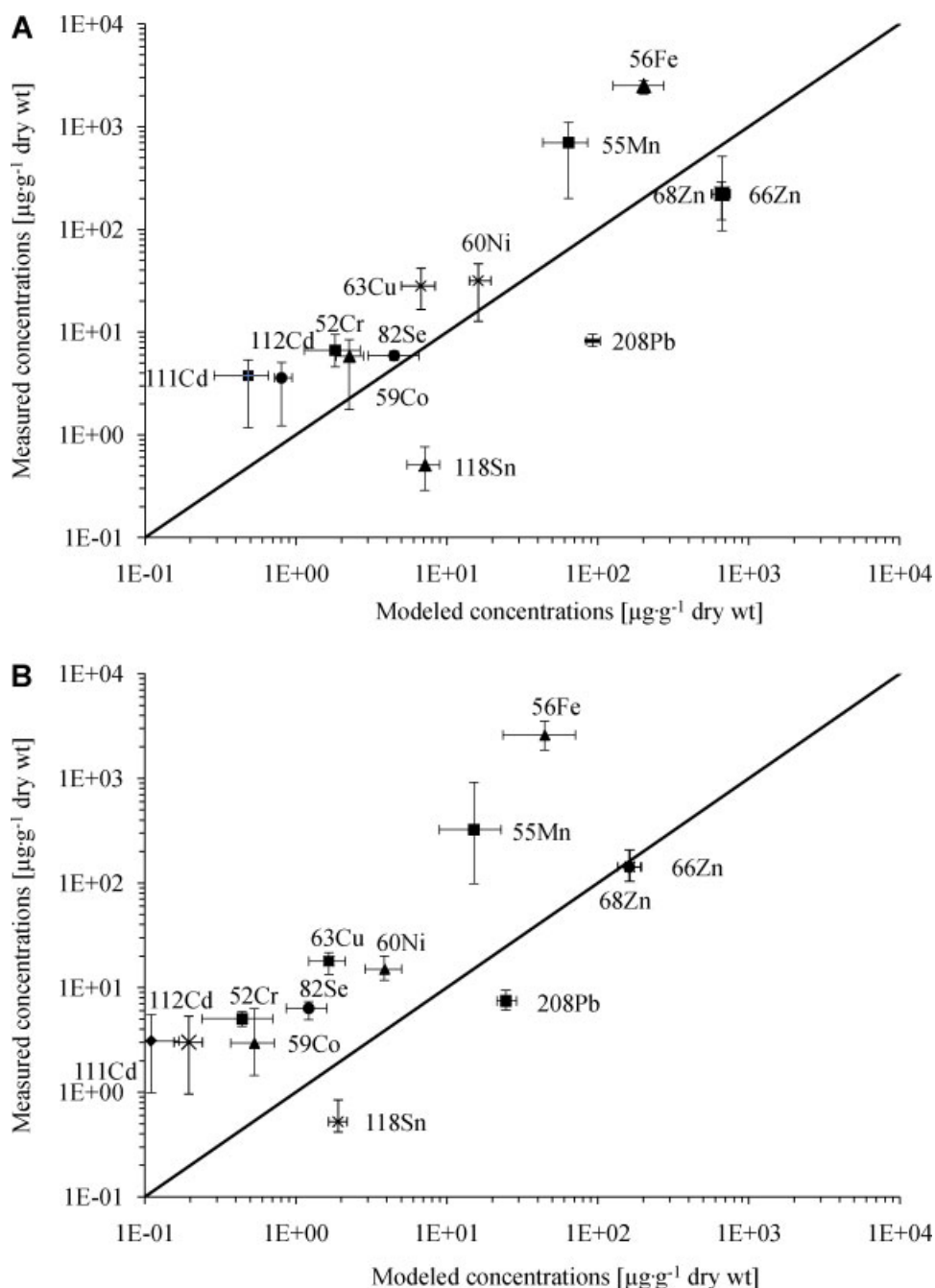


Fig. 1. Comparison of modeled and measured metal concentrations in zebra mussels (A) and in quagga mussels (B) from the rivers Rhine and Meuse, the Netherlands.

Bioconcentration levels were highest for  $^{82}\text{Se}$  and  $^{208}\text{Pb}$  and lowest for  $^{95}\text{Cr}$  in both species. The BCF and bioaccumulation factors values of zebra mussels were significantly higher than those of quagga mussels ( $p < 0.0001$ ).

## DISCUSSION

### *Metal bioaccumulation in zebra and quagga mussels*

Metal concentrations in mussels from the river Rhine were generally lower than those in the river Meuse. Similar results were found in a previous study by Hendriks et al. [31] for Cd and Zn, but not for the other metals (Table 1). This change may be attributable to a more significant improvement in water quality in the river Rhine. Metal concentrations in the zebra

mussels taken from the rivers Rhine and Meuse were in the range reported for the Lawrence River [32], except for  $^{55}\text{Mn}$  and  $^{208}\text{Pb}$ , with higher levels found in the present study (Table 1). The level of bioaccumulation from food modeled in the present study was lower than that derived by Roditi et al. [14]. This may be attributed to differences in food items included. These authors modeled bioaccumulation from total particulate metal concentrations (labile and refractory fraction) while we integrated metal uptake from phytoplankton.

The relative importance of uptake from the dissolved phase and from food, as reported in the literature, is inconclusive. DeForest et al. [33] found inverse relations between the BCF and bioaccumulation factors and water concentrations, indicating a complex relationship between absorption and ingestion

Table 1. Average metal concentrations in zebra (*Dreissena polymorpha*) and quagga (*Dreissena rostriformis bugensis*) mussels in the rivers Rhine and Meuse (average  $\pm$  standard deviation,  $\mu\text{g/g}$  dry wt)

Metals	Zebra mussels					Quagga mussels	
	Rhine	Meuse	Rhine <sup>a</sup>	Meuse <sup>a</sup>	Lawrence River	Rhine	Meuse
<sup>52</sup> Cr	4.60	7.68 $\pm$ 2.60	5.00	2.73	0.46–9.45	4.98 $\pm$ 0.84	5.10 $\pm$ 0.55
<sup>59</sup> Co	1.76	7.88 $\pm$ 0.77				1.68 $\pm$ 0.21	4.23 $\pm$ 1.81
<sup>66</sup> Zn	124.41	269.52 $\pm$ 30.99	241.67	418.18	129–340	114.78 $\pm$ 10.22	170.82 $\pm$ 32.70
<sup>68</sup> Zn	123.76	269.32 $\pm$ 20.48				114.30 $\pm$ 10.29	170.06 $\pm$ 32.98
<sup>82</sup> Se	5.26	6.23 $\pm$ 0.46			4.05–7.4	5.69 $\pm$ 0.69	6.99 $\pm$ 0.34
<sup>111</sup> Cd	1.18	5.06 $\pm$ 0.45				1.12 $\pm$ 0.12	5.04 $\pm$ 0.43
<sup>112</sup> Cd	1.22	4.76 $\pm$ 0.42	1.33	3.86	1.8–7.43	1.11 $\pm$ 0.13	4.90 $\pm$ 0.41
<sup>208</sup> Pb	7.23	8.72 $\pm$ 1.15	3.92	2.91	0.31–1.78	6.75 $\pm$ 0.59	8.16 $\pm$ 1.18
<sup>55</sup> Mn	200.89	947.33 $\pm$ 214.21	158.33	81.82	35–96	165.87 $\pm$ 68.64	482.40 $\pm$ 379.90
<sup>56</sup> Fe	2077.69	2,721.77 $\pm$ 88.95				2,107.57 $\pm$ 241.09	3,091.31 $\pm$ 384.24
<sup>60</sup> Ni	12.68	41.01 $\pm$ 7.17	20.83	10.00	8.84–55.2	11.95 $\pm$ 0.19	18.08 $\pm$ 2.32
<sup>63</sup> Cu	16.59	33.93 $\pm$ 10.94	22.50	17.27	14.2–35.9	17.91 $\pm$ 4.15	17.92 $\pm$ 2.78
<sup>118</sup> Sn	0.76	0.38 $\pm$ 0.14				0.62 $\pm$ 0.20	0.42 $\pm$ 0.01
Study	Present study	Present study	[31]	[31]	[32]	Present study	Present study

<sup>a</sup>Secondary data from Hendriks et al. [31].

The studies by Hendriks et al. [31] and Kwan et al. [32] do not mention isotopes of Cd and Zn.

with metal levels in water and food. The metal-specific relative contribution of the two sources to tissue accumulation found in this study is consistent with results from some other studies [12–14]. The present study confirmed the findings by Mersch et al. [34] that cadmium concentrations in zebra mussels were mainly determined by exposure to the aqueous phase.

#### Uncertainties

Some assumptions included in our model resulted in uncertainties. Important sources of uncertainties are related to food items, filtration rate, the BCF of phytoplankton, and kinetic rate constants. These factors are discussed in the following sections.

**Food items.** Mussels can consume a variety of food items, ranging from phytoplankton to bacteria, detritus, and small zooplankton. According to Bruner et al. [35], uptake via algae is more important at the same exposure concentrations of algae and suspended particles. However, the contribution of algae and suspended sediment to metal bioaccumulation is determined by both the metal concentrations in these food items as well as their availability. Zebra mussels are able to filter particles as small as 0.7  $\mu\text{m}$ , and the maximum retention efficiency is obtained at sizes larger than 5  $\mu\text{m}$  [18]. In the Lower Rhine, small centric diatoms with sizes that are effectively cleared by mussels were the major component of the algal community (LWA 1989–1993). Moreover, shell growth rates were strongly related to the chlorophyll *a* concentration, indicating phytoplankton as an important food source [29]. In contrast, the mussel growth was found not to be correlated to the dissolved organic matter, total organic content, or biomass of seston of the river Rhine water [36]. Similarly, no correlations were found between biomass of bacterial populations and chlorophyll *a* concentrations (LWA, 1988). These results may indicate that the fraction of bacteria in zebra mussel food is insignificant [29].

**Filtration rate.** Filtration rate is an important physiological parameter, determining uptake from both water and food. Filtration is related to food selectivity and pseudofeces production, which were excluded in the present model. Similar filtration rates were found for different types of food as well as for various phytoplankton taxa in different sizes, suggesting that the influence of particle selection is negligible [18,20,37]. The exclusion of food selectivity in terms of size and types is therefore expected not to cause large uncertainties. Another factor influencing the filtration rate is pseudofeces production

suggested to clear excess particles or to reject some particle types [18]. Clearing excess particles only occurs when food concentrations exceed a certain level, the so-called incipient limiting concentration. Different values of this concentration have been reported, but all were substantially higher than food levels measured in the rivers Rhine and Meuse [18,22]. Although rejection of particles by pseudofeces production is known to occur even at low food concentrations [18], almost complete retention efficiency was reported for algae, the main food source in the present study [22,26]. As a result, uncertainties from the exclusion of the pseudofeces production in our model were assumed to be insignificant.

**BCF of phytoplankton.** The bioconcentration factor of phytoplankton, BCF<sub>p</sub>, determines metal concentrations in food. But its value may decrease with the exposure concentrations as reported by Hendriks and Heikens [27]. This was not integrated in our approach to keep the model simple. This simplification was justified by choosing BCF<sub>p</sub> values obtained at similar levels of metal exposure as in our sampling sites.

**Kinetic rate constants.** In the present model, metal absorption and assimilation efficiencies as well as elimination and growth rates were considered independent of environmental conditions, particularly metal and food exposure concentrations. This assumption may not be completely justified in all cases. In addition, the physiological processes may be influenced by metal-specific biological regulations by mussels. For example, essential metals, such as Cu and Zn, can be taken up at high amounts, and their tissue concentrations can be regulated biologically [38]. However, measured concentrations of these essential metals did not deviate more from modeled levels than noted for other metals (Fig. 1). In addition, in the present study, the covalent index was used to model metal absorption efficiency and elimination rates. Yet, metal bioaccumulation is also related to other chemical properties, allowing further improvement of the estimations. For example, atomic weight was shown to considerably contribute to variations in metal tissue concentrations [31].

#### Recommendations

Validation in the present study showed the good potential of the model in estimating metal concentrations in zebra and quagga mussels. As noted by Veltman et al. [8], integration of the covalent index thus may significantly improve modeling

of metal bioaccumulation. By integrating this metal-specific property and the size-based filtration rate, metal bioaccumulation can be predicted for a number of metals without calibration for specific cases. Moreover, the difficulties and limitations in the application of bioaccumulation in metal risk assessment can be overcome because physiological processes influencing metal uptake kinetics can be included.

However, caution should be taken in applying the model, because assumptions that apply to the rivers Rhine and Meuse may not hold in other water systems. The estimation potential of the model can be improved by considering some additional factors, specifically, dependence of physiological rate constants on exposure concentrations, other chemical properties, such as molecular weight, and metal-specific behavioral characteristics of mussels, for example, biological internal regulations and sequestration.

#### SUPPLEMENTAL DATA

Tables S1–S10. (166 KB DOC).

**Acknowledgement**—We thank J. Eygensteyn, M. Orbons, A. Schipper, and anonymous reviewers.

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