

## Rates and routes of trace element uptake in zebra mussels

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### Abstract

The zebra mussel, *Dreissena polymorpha*, a nonindigenous invasive species, is now widespread throughout the eastern half of North America. Because zebra mussels are ubiquitous and because they effectively filter particulate matter out of suspension, the cycling and residence times of particle-reactive metals will likely be affected in waters with zebra mussels. This study describes experiments designed to assess the possibility of using this species as a bioindicator of metals in ambient freshwater environments. Laboratory exposures of zebra mussels to <sup>110m</sup>Ag, <sup>109</sup>Cd, <sup>51</sup>Cr, <sup>14</sup>C, <sup>203</sup>Hg, and <sup>75</sup>Se were employed to measure their assimilation efficiencies (percentage of ingested element that crosses gut lining) from eight food types (four algal species and bacteria, seston, and mineral assemblages), absorption efficiencies from water (percentage of element pumped by the mussel that is absorbed by the animal), and rates of depuration of these elements from mussels following long-term exposures to food and water. Assimilation efficiencies of elements from foods ranged from 4 to 29% for silver (Ag), 19 to 72% for cadmium (Cd), 42 to 85% for carbon (C), 2 to 19% for chromium (Cr), 4 to 40% for mercury (Hg), and 8 to 46% for selenium (Se). Absorption efficiencies from the dissolved phase were 1.87% for Ag, 1.02% for Cd, 0.47% for Cr(III), 0.27% for Cr(VI), 1.17% for Hg, and 0.03% for Se. Efflux rate constants (d<sup>-1</sup>) following long-term exposure to food and water were 0.067 and 0.084 for Ag, 0.013 and 0.011 for Cd, 0.019 and 0.011 for Cr, 0.050 for Hg (food only), and 0.026 and 0.035 for Se. These loss rates corresponded to biological half-lives ranging from 8 d for Ag to 76 d for Se. Loss rates of trace elements from zebra mussel feces followed the following sequence: Cr < Ag < Se < Hg ≤ Cd, with average retention half-times being 59, 43, 11, 6.7, and 5.1 d, respectively, which indicates that geochemical cycling rates from zebra mussel biodeposits are element specific. Egestion patterns of the radioisotopes indicated two digestive phases, extracellular and intracellular digestion. The extent of intracellular digestion ranged from 7 to 40%, depending on the food source, and correlated with assimilation efficiency for Ag, Cd, and Hg. The bioaccumulation parameters measured for *D. polymorpha* can be used in kinetic models to quantify the relative importance of food and water as sources of metals and to predict on a site-specific basis the tissue concentrations of metals in these mussels, as shown for Cd. Because *D. polymorpha* accumulates metals from dissolved and particulate sources in proportion to ambient concentrations, this species can be an effective bioindicator of freshwater metal contamination.

Many trace elements of environmental concern are particle reactive and concentrate on suspended particles, including phytoplankton, in aquatic systems. The introduction of zebra mussels (*Dreissena polymorpha*) to many regions of North America may lead to a potential change in the flux of many trace elements in these waters because these mussels are so effective in grazing on phytoplankton and seston in lakes and rivers (Fahnenstiel et al. 1995; Caraco et al. 1997). However, few studies have investigated changes in trace element cycling in waters with zebra mussels. Individual zebra mussel pumping rates and particle ingestion rates (Roditi et al. 1996) scaled to common population densities suggest that a high fraction of the total load of a particle-reactive trace element in a body of water will pass through zebra mussels, either through the gut or through the mussels' gills in the dissolved phase.

Furthermore, given their current and anticipated wide-

spread distributions (Strayer and Smith 1993) and high grazing rates, zebra mussels may be effectively used as bioindicators of freshwater contamination, much like the marine mussel *Mytilus edulis*, which has been used to monitor coastal water quality. Toward this end, it is necessary to quantify the relationship between ambient contaminant concentrations and mussel concentrations and in particular to measure the sources of contaminants (food versus water) for these animals. Until recently, there had not been a freshwater counterpart in North America to the coastal marine Mussel Watch component of the National Status and Trends (NS&T) program, despite the fact that freshwater food webs may be vulnerable to metal contamination due to such factors as the absence of many competing ions and complexing ions such as Cl<sup>-</sup>. Recognizing this, in 1992 the NS&T program added the zebra mussel to its sampling program at seven sites along the Great Lakes and at one site on the Hudson River.

Bioenergetic-based kinetic models have been developed to evaluate the bioaccumulation of contaminants in aquatic animals, including mussels (Wang et al. 1996). These models require information on uptake efficiencies from food and water pathways (physiological assimilation and absorption efficiencies), depuration rates of contaminants from the animals, and filtration, ingestion, and growth rates, and can be solved for steady-state tissue concentrations under any set of environmental conditions. These models are therefore very flexible and applicable to diverse environments with varying

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conditions. They can be useful in interpreting monitoring data and can be used to predict contaminant levels in animals on a site-specific basis.

Previous research has recognized the zebra mussel's potential as a bioindicator organism to assess spatial and temporal trends in concentrations of bioavailable contaminants (Kraak et al. 1991; Mersch et al. 1992). However, there have been limitations to using this research for quantitatively describing bioaccumulation in these mussels and their influence on geochemical cycling. For example, the relative bioavailability of different trace element sources (food and water) for zebra mussels has never been evaluated. Studies that exposed mussels to trace elements in a suspension of natural seston (Klerks and Fraleigh 1997) may have simulated a specific natural system well, but if the food and water uptake pathways are not investigated quantitatively and independently of one another, it is not possible to model bioaccumulation or apply these results to other systems with differing seston quality and quantity. There is also difficulty with studies exposing mussels to dissolved trace elements only in which it is assumed that bioconcentration factors are constant (Bias and Karbe 1985). Bioconcentration factors have been shown to vary from one site to another (Mersch et al. 1992), probably depending on local total suspended solids (TSS) characteristics and other parameters such as dissolved organic carbon, water hardness, sulfide and chloride concentrations, and pH. Although consideration has been given to monitoring freshwater concentrations of trace elements using zebra mussels, no study has quantified the relative importance of different uptake pathways of trace elements into zebra mussels, and kinetic parameters that may assist in the development of a bioaccumulation model for contaminants in these animals have not been evaluated.

We therefore conducted a series of experiments to determine metal assimilation efficiencies in zebra mussels from different food types (and the physiological factors that influence them), absorption efficiencies of dissolved trace elements, and long-term efflux rates of trace elements following exposure to particulate and dissolved sources. We also determined the flux of trace elements out of zebra mussel fecal material to ambient water, as this could influence the geochemical cycling of these trace elements. The trace elements examined in this study—silver, cadmium, chromium, mercury, and selenium (Ag, Cd, Cr, Hg, and Se)—are of environmental concern in many inland (and coastal) waters, often because of industrial activity. These elements, which can be toxic at elevated concentrations, are regularly monitored in coastal waters using marine bivalves. Results from this study can also be used to evaluate the potential of using zebra mussels as quantitative bioindicators of these metals in freshwater systems.

Use of gamma-emitting radiotracers provided this work with several advantages, including the ability to monitor individual mussels nondestructively over time, thereby reducing biological variability. Pulse-chase techniques kept feeding periods shorter than gut passage times, thereby making it possible to measure total ingestion, determine assimilation efficiencies, and reduce recycling within experiments. Pulse-chase methods also reduced desorption during feedings and

reduced behavioral artifacts associated with longer experiments.

## Materials and methods

*Mussel and water collection*—Zebra mussels (18–22-mm shell length; mean dry weight of soft parts =  $16.6 \pm 3.4$  mg) were collected from the tidal freshwater portion of the Hudson River (Poughkeepsie and Tivoli, New York) and acclimated in the lab on a diet of *Chlorella* powder (Nichols 1993) at 15°C for at least 1 week. Hudson River water was collected at the same locations, stored at 4°C, and filtered through Millipore 0.22- $\mu\text{m}$  Millistak cartridges immediately before experiments. All experiments were conducted at the acclimation temperature of 15°C.

*Particle concentration factors*—Algal cell cultures were counted and cell sizes measured with a Coulter Multisizer II particle analyzer; cell and seston dry weights were determined by filtering cells on tared Nuclepore membranes and weighing them after drying at 70°C for 48 h. Bacteria were counted by epifluorescence and sized by stage micrometer. Bacteria cell volumes and dry weights were estimated following Bratbak and Dundas (1984). Particle concentration factors by volume (VCF) were calculated for each radioisotope as  $\text{VCF} = (\text{atoms } \mu\text{m}^{-3} \text{ cell volume}) / (\text{atoms } \mu\text{m}^{-3} \text{ water, dissolved})$ , and by dry weight (DCF) as  $\text{DCF} = (\text{atoms } \text{pg}^{-1} \text{ cell dry weight}) / (\text{atoms } \text{pg}^{-1} \text{ water, dissolved})$ .

*Trace element and C assimilation from food in D. polymorpha*—The assimilation of trace elements from different particulate sources was determined using unialgal cultures representing the major algal groups in the Hudson River and other particles. Food particles included *Thalassiosira pseudonana* (small centric diatom), *Cyclotella meneghiniana* (large centric diatom), *Chlorella vulgaris* (chlorophyte), *Microcystis aeruginosa* (cyanophyte), a Hudson River bacteria assemblage, two Hudson River seston assemblages, and an assemblage of mineral particles. Cell dimensions and weights are given in Table 1.

Algae were cultured in WC freshwater medium (Guillard 1975) on a 14:10 light:dark cycle at 15°C. The pH of the medium was adjusted to 7.0. Cells in late log phase were removed from stock cultures and resuspended in WC medium modified to include one-tenth trace metals concentration and no addition of copper (Cu), zinc (Zn), or ethylenediaminetetraacetic acid (EDTA). These cultures were usually radiolabeled in the following combinations:  $^{110\text{m}}\text{Ag}$  (in 0.1 N  $\text{HNO}_3$ ) +  $^{109}\text{Cd}$  (in 0.1 N HCl) +  $^{75}\text{Se}$  ( $\text{Na}_2\text{SeO}_3$  in distilled water),  $^{14}\text{C}$  ( $\text{NaHCO}_3$  in distilled water, except for the bacteria assemblage, which was D-[U- $^{14}\text{C}$ ] glucose in distilled water) +  $^{51}\text{Cr}$  (in 0.1 N HCl), and  $^{203}\text{Hg}$  (in 1 N HCl) separately. Prior to radioisotope additions, small volumes of NaOH were added to neutralize acidic radioisotope additions. Radioisotope additions to culture media were within the following concentrations: 4–21 kBq  $\text{L}^{-1}$  (3–166 nM) for  $^{110\text{m}}\text{Ag}$ , 9–211 kBq  $\text{L}^{-1}$  (1–26 nM) for  $^{109}\text{Cd}$ , 28–99 kBq  $\text{L}^{-1}$  (0.6–7 nM) for  $^{203}\text{Hg}$ , 63–214 kBq  $\text{L}^{-1}$  (4–13 nM) for  $^{75}\text{Se}$ , and 230–1233 kBq  $\text{L}^{-1}$  (1–16 nM) for  $^{51}\text{Cr}$ .

Bacteria assemblages were obtained from 1.0- $\mu\text{m}$  (Nucle-

Table 1. Algal species, bacteria assemblages, and seston assemblages used in feeding experiments, cell dry weights and diameters (volumes for bacteria), metal concentration factors and metal concentrations on particles.\*

Species	Dry wt (pg cell <sup>-1</sup> )	Diameter (μm)	Metal	VCF (× 10 <sup>4</sup> )	DCF (× 10 <sup>4</sup> )	μmoles g <sup>-1</sup> dry wt cells
<i>C. vulgaris</i> (chlorophyte)	12	2.8	Ag	2.0	2.0	1.58
			Cd	4.5	4.5	0.33
			Cr	1.0	1.0	0.28
			Se	0.03	0.03	0.0007
<i>C. meneghiniana</i> (diatom)	135	7.1	Ag	1.2	1.6	0.35
			Cd	1.6	2.1	0.12
			Cr	3.1	4.2	0.04
			Se	1.8	2.4	0.02
<i>M. aeruginosa</i> (cyanophyte)	14	3.9	Ag	2.6	5.5	2.02
			Cd	5.2	11.3	0.34
			Cr	0.8	1.7	0.15
			Se	0.04	0.08	0.001
<i>T. pseudonana</i> (diatom)	30	4.7	Ag	2.8	4.9	1.81
			Cd	5.1	9.2	0.21
			Cr	10.6	19.0	0.03
			Se	3.0	5.4	0.02
Bacteria assemblage Ag + Cd + Se Cr + C	0.1 0.3	0.2 μm <sup>3</sup> 0.6 μm <sup>3</sup>	Ag	0.4	0.9	0.57
			Cd	0.1	0.2	0.02
			Cr	1.4	3.1	0.01
			Se	0.02	0.04	0.002
Seston assemblage A	ND	ND	Ag	ND	3.1	2.58
			Cd	ND	1.5	0.21
			Cr	ND	5.7	0.53
			Se	ND	0.02	0.003
Seston assemblage B	ND	ND	Ag	ND	18.1	0.16
			Cd	ND	8.3	0.05
			Hg	ND	11.4	0.03
			Se	ND	0.2	0.005
Mineral assemblage	ND	ND	Ag	ND	1.6	0.04
			Cd	ND	0.19	0.004
			Cr	ND	3.8	0.02
			Hg	ND	1.3	0.07
			Se	ND	0.01	0.0006

\* VCF, particle concentration factors by volume (metal μm<sup>-3</sup> cell)/(metal μm<sup>-3</sup> dissolved in water)<sup>-1</sup>; DCF, particle concentration factors by dry weight = Kd = (metal g<sup>-1</sup> dry wt cell)/(metal ml dissolved in water)<sup>-1</sup>; ND, not determined.

pore polycarbonate membranes) filtrates of Hudson River water. WC freshwater medium supplemented with 100 mg L<sup>-1</sup> glucose was inoculated with 10 mL of bacterial filtrate and spiked as above, except <sup>14</sup>C was added as D-[U-<sup>14</sup>C]glucose. Cultures were kept at room temperature (18°C) on a shaker table for 6–12 d and collected by centrifugation (7,000 × g for 5 min, then supernatant again at 10,000 × g for 10 min), then sonicated at low energy. Cells from the <sup>14</sup>C + <sup>51</sup>Cr treatment only were then filtered through a 1-μm membrane, and only the filtrate (<1 μm) was used in the feeding experiment. To reach targeted feeding concentrations, light absorbance of the bacterial suspension was mea-

sured at 660 nm and converted to corresponding suspended dry weight using a simple empirical relationship.

Prior to radiolabeling, Hudson River seston (assemblage A collected 20 July 1996 and assemblage B collected 20 July 1998) was analyzed for TSS load and particulate carbon (C) and nitrogen (N) (CHN analyzer). The TSS load of A was 28 ± 0.6 mg L<sup>-1</sup>, and the load of B was 11 ± 0.3 mg L<sup>-1</sup>; the particulate organic C and particulate organic N concentrations of A were 0.74 ± 0.02 and 0.05 ± 0.004 mg L<sup>-1</sup>, respectively, and the concentrations of B were 0.48 ± 0.02 and 0.03 ± 0.002, respectively (mean ± SE; n = 4). A 250-ml aliquot was spiked and incubated at room temperature (18°C) for 60 h at low light (10 μEin m<sup>-2</sup> s<sup>-1</sup>). Radiolabeled seston was collected on a 0.2-μm polycarbonate membrane and resuspended in 0.2-μm filtered Hudson River water. To examine metal assimilation from inorganic material, an assemblage of mineral particles was created from quartz, k-feldspar, and illite clay, all of which are important components of Hudson River suspended matter (Olsen et al. 1978). Minerals were ground to a fine powder and resuspended (32 mg L<sup>-1</sup>) in equal proportion in 0.2-μm filtered Hudson River water, incubated 48 h, then radiolabeled for 60 h and harvested as described above.

C assimilation in mussels was measured by the <sup>14</sup>C: <sup>51</sup>Cr ratio method (Calow and Fletcher 1972), which assumes Cr is an inert tracer of digestion. The following equation was used:

$$AE (\%) = [1 - (^{14}\text{C}:^{51}\text{Cr})_{\text{feces}} / (^{14}\text{C}:^{51}\text{Cr})_{\text{food}}] \times 100, (1)$$

in which (<sup>14</sup>C: <sup>51</sup>Cr)<sub>feces</sub> is the ratio of cumulative <sup>14</sup>C to <sup>51</sup>Cr radioactivity in feces at 72 h of depuration, calculated individually for each mussel, and (<sup>14</sup>C: <sup>51</sup>Cr)<sub>food</sub> is the ratio of <sup>14</sup>C to <sup>51</sup>Cr radioactivity on particles during the radioactive feeding. This approach for determining assimilation efficiency (AE) of ingested C assumes that all food particles are equally labeled with <sup>14</sup>C and <sup>51</sup>Cr. While this equation assumes that <sup>51</sup>Cr is inert to mussels, it was found that some <sup>51</sup>Cr was assimilated (Table 2), and the term (<sup>14</sup>C: <sup>51</sup>Cr)<sub>feces</sub> thus required correction for <sup>51</sup>Cr remaining in tissues, which was done by adding the <sup>51</sup>Cr activity remaining in tissues at 72 h to the <sup>51</sup>Cr term of (<sup>14</sup>C: <sup>51</sup>Cr)<sub>feces</sub>. Feces were collected with a Pasteur pipet within 15 min of production during the first 12 h of depuration and every few hours thereafter. It was assumed that <sup>14</sup>C: <sup>51</sup>Cr ratios in the fecal pellets did not change between time of defecation and time of pellet collection. Fecal pellets were first gamma-counted for <sup>51</sup>Cr emissions (see below), then solubilized (Solvable, Packard), placed in scintillant (Ultima Gold XR, Packard), and re-counted (Packard Tri-Carb 2100-TR Liquid Scintillation Analyzer). Because <sup>51</sup>Cr interferes with <sup>14</sup>C counting, <sup>14</sup>C samples were counted after a minimum of six <sup>51</sup>Cr half-lives (t<sub>1/2</sub> = 27.7 d) elapsed to reduce interference. Quenching was corrected using an external standard method.

**Radioactive feeding and depuration**—Cells were considered uniformly labeled after undergoing at least three cell divisions and were collected on 1.0-μm membranes and resuspended into 50-ml volumes of unlabeled deionized water, then pipetted from these concentrated suspensions into beakers containing between 0.5 to 1.0 liter of 0.2-μm filtered

Table 2. Element assimilation efficiencies (%) in *D. polymorpha* from eight radiolabeled foods. Values given as means  $\pm$  1 SE;  $n = 5$  for all treatments except bacteria  $^{14}\text{C} + ^{51}\text{Cr}$  treatment for which  $n = 7$ . ND, not determined.

Element	C.		T.	M.	Bacteria assemblage	Seston A	Seston B	Mineral assemblage
	<i>C. vulgaris</i> (chloro-phyte)	<i>meneghiniana</i> (large diatom)	<i>pseudonana</i> (small diatom)					
Cd	22 $\pm$ 2	26 $\pm$ 2	48 $\pm$ 4	72 $\pm$ 4	56 $\pm$ 0	19 $\pm$ 1	26 $\pm$ 2	20 $\pm$ 1
Se	18 $\pm$ 1	24 $\pm$ 3	46 $\pm$ 3	40 $\pm$ 4	41 $\pm$ 1	7.7 $\pm$ 0.4	23 $\pm$ 2	28 $\pm$ 3
Ag	3.9 $\pm$ 2.0	4.0 $\pm$ 0.4	7.4 $\pm$ 0.9	16 $\pm$ 3	9.5 $\pm$ 2.8	5.5 $\pm$ 0.9	4.3 $\pm$ 0.6	29 $\pm$ 1
Cr	6.3 $\pm$ 1.3	5.0 $\pm$ 0.8	5.0 $\pm$ 0.5	4.7 $\pm$ 1.2	19 $\pm$ 1	1.5 $\pm$ 0.5	ND	2.1 $\pm$ 0.6
C	42 $\pm$ 6	ND	81 $\pm$ 5	85 $\pm$ 1	46 $\pm$ 5	58 $\pm$ 1	ND	ND
Hg	13 $\pm$ 2	ND	21 $\pm$ 3	40 $\pm$ 3	ND	ND	3.6 $\pm$ 0.6	8.5 $\pm$ 1.4

Hudson River water with 5–7 zebra mussels. Particle concentrations in unialgal feedings were low, ranging from 1.0 to 3.0 mg L<sup>-1</sup>; seston assemblages were adjusted to either 4 or 14 mg L<sup>-1</sup>; mineral assemblages were adjusted to 8 mg L<sup>-1</sup>. Feeding time was 30 min, which was less than the observed gut passage time, to avoid defecation of radiolabel and recycling of trace elements during the radiolabeled feeding. Radiolabeled cells were added at 10-min intervals to maintain constant cell concentrations. After feeding, mussels were removed, rinsed, and radioassayed nondestructively (described below). Counting times were generally 5 min, after which they were placed individually within 240-ml polypropylene beakers containing 150 ml of 0.2- $\mu\text{m}$  filtered Hudson River water, within a recirculating system containing 15 liters of 0.2- $\mu\text{m}$  filtered Hudson River water (Wang et al. 1995). Flow through the beakers was maintained at about 2 L h<sup>-1</sup>. The radioactivity of these mussels during their depuration periods was monitored periodically over time.

During feeding, desorption of trace elements was monitored by filtering aliquots of the feeding suspension at 10-min intervals and calculating the fraction of each radioisotope on particles (Fisher et al. 1983). The contribution of desorbed radioisotope to uptake in the mussels was determined in some particle treatments by removing the particles remaining after feeding (1.0- $\mu\text{m}$  filtration), placing 2 to 3 “control” mussels in the filtrate for 30 min, and counting the radioactivity of these control mussels. In all cases, ingested food was the dominant source of radioisotope for the mussels, with control mussels having a mean of 0.3% for Ag, 2.7% for Cd, 0.1% for Cr, 0.03% for Hg, and 3.0% for Se of the radioactivity of the fed mussels.

Throughout the depuration periods, feces were collected from individual mussels as quickly as possible after production and radioassayed. Little radioisotope was found in feces produced  $\sim$ 72 h after the onset of depuration, which indicates that digestion and trace element assimilation was completed within this time (Fig. 1). Feces were collected most frequently during the first 12 h (every 30 min) to monitor egestion at the time most radioactive debris was egested and to prevent loss of radioisotopes from feces to the ambient water. After depuration, mussels were dissected to measure radioactivity in shell and soft parts. AE was defined as the percentage of ingested trace element retained in the mussels after complete digestion (usually 72 h). Gut passage time of a metal is defined as the time required for ingested metal to

be defecated and is calculated for individual metals as the time needed for 90% of the total metal egested to be recovered in feces (Wang and Fisher 1996).

*Filtration rates of suspended particles*—To monitor feeding rates during radioactive feeding, especially for the  $^{14}\text{C} + ^{51}\text{Cr}$  bacteria treatment for which all particles were  $<1 \mu\text{m}$  and for which zebra mussel feeding efficiencies were unknown to us, we used the formula:

$$\text{FR} = [\text{DPM}_{\text{whole animal}} / (\text{DPM}_{\text{particulate}} \text{ ml}^{-1})] \times 2, \quad (2)$$

where FR is the filtration rate (ml h<sup>-1</sup>),  $\text{DPM}_{\text{whole animal}}$  is disintegrations min<sup>-1</sup> of a radioisotope in a whole mussel immediately following the 30-min radioactive feeding period, and  $(\text{DPM}_{\text{particulate}} \text{ ml}^{-1})$  is disintegrations min<sup>-1</sup> of radioisotope on particles in 1 ml of feeding water; the fraction  $\text{DPM}_{\text{whole animal}} / (\text{DPM}_{\text{particulate}} \text{ ml}^{-1})$  is multiplied by two to normalize our 30 min measurements to an hourly basis. This method is accurate only if  $\text{DPM}_{\text{particulate}} \text{ ml}^{-1}$  remains constant in the feeding water (i.e., constant particle load and no desorption). FRs were calculated only for particle treatments in which  $\text{DPM}_{\text{particulate}} \text{ ml}^{-1}$  remained relatively constant and for  $^{14}\text{C} + ^{51}\text{Cr}$  treatments, because  $^{51}\text{Cr}$  desorbed less than other trace elements. Note that assimilation efficiencies are irrelevant here because the  $\text{DPM}_{\text{whole animal}}$  is measured immediately after feeding and prior to any release of fecal material.

*Release of trace elements from mussel feces*—The rate of trace element cycling through feces was determined by incubating freshly collected radioactive fecal pellets in beakers containing 50 ml of overlying 0.2- $\mu\text{m}$  filtered Hudson River water, following the protocol of Fisher et al. (1991). Overlying water was periodically removed gently by pipet from unstirred vessels and fecal pellets minus overlying water were radioassayed, after which 50 ml of fresh 0.2- $\mu\text{m}$  filtered Hudson River water was added to the fecal pellets, at which time pellets were stirred up but remained intact, and the incubation continued. Periodically, aliquots of overlying water removed by pipet were filtered using 0.2- $\mu\text{m}$  Nucleopore membranes to check for removal of fecal pellets (or parts of pellets) with overlying water; in all cases, this was negligible.

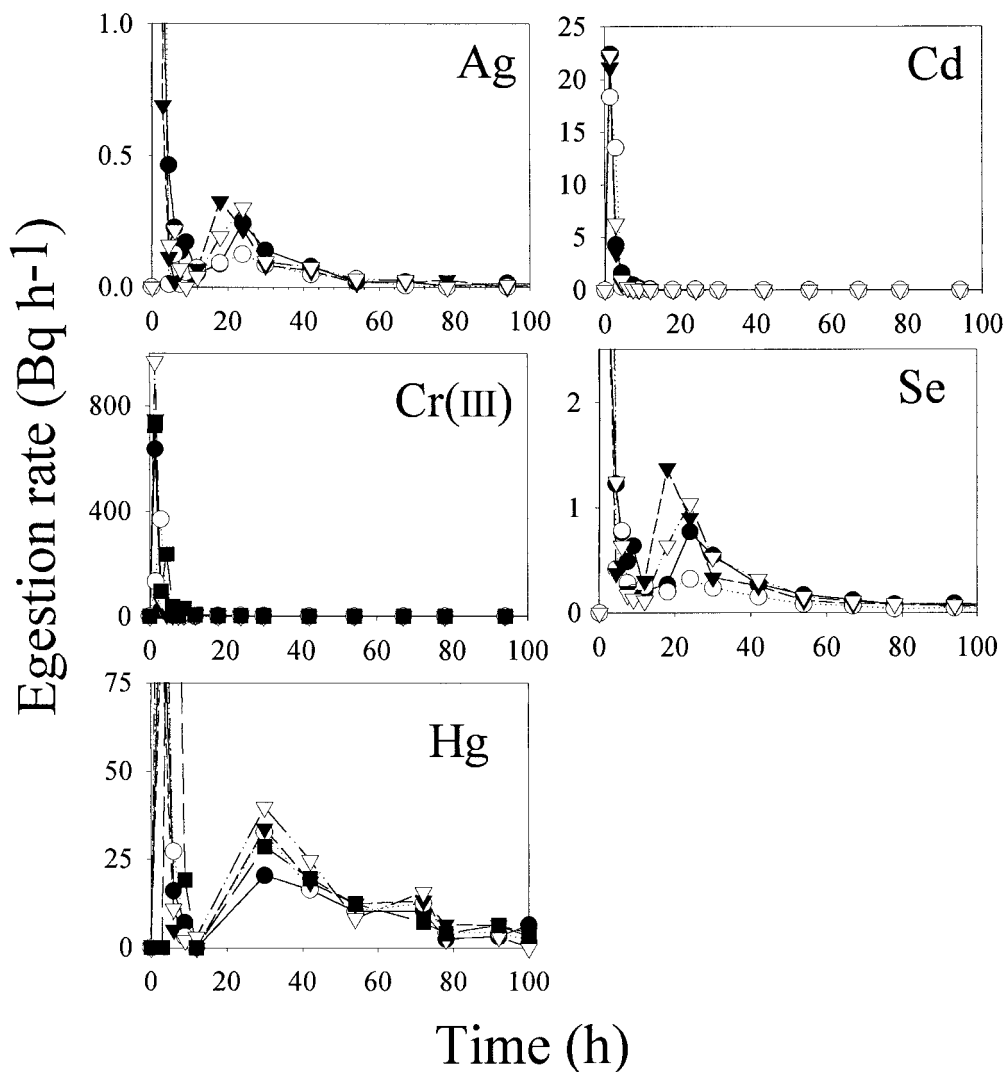


Fig. 1. Egestion rates of radioisotopes following 30 min pulse feeding. Fecal pellets were collected from individual mussels at regular intervals, and their radioactivity was counted. The radioactivity was divided by the time interval of collection to yield an egestion rate. Each data series and symbol represents an individual mussel. Note that silver, mercury, and selenium have a second egestion maximum indicative of intracellular digestion (Y-axis scale is adjusted to show peaks for each metal).

*Trace element influx rates from the dissolved phase*—Availability of dissolved trace elements to zebra mussels was determined by exposing mussels to trace elements in solution. Measuring uptake of dissolved trace elements in bivalves can be complicated by the reduction of pumping rates in the absence of particles, thus leading to reduced influx rates. To limit such a decline and reduce artifacts resulting from efflux from mussels during exposures, these experiments were limited to 2 h. For the marine mussel *Mytilus edulis*, pumping rate in the absence of particles does not decline appreciably in the first 2 h (Widdows and Hawkins 1989). Visual observations indicated that the experimental zebra mussels continued to pump normally over the 2-h exposure period.

Mussels were placed in beakers with 0.6 liters of 0.2- $\mu\text{m}$  filtered Hudson River water, pH 7.3, with a range of trace

element concentrations (mixtures of stable and radioisotopes, except for the lowest concentrations of Ag and Se, which contained only radioisotope). Trace element exposures were to one element only; multiple-isotope exposures were not used. Mussels were exposed to each element at a range of concentrations, and each concentration included seven replicate mussels with shell lengths between 19 and 22 mm. Equal amounts of radioisotope were added to all beakers, and stable metals were added to produce a 2 order of magnitude range of concentrations for each element. Lowest concentrations were between  $\sim 1$  and 10 times ambient freshwater concentrations, as determined from averages of published and unpublished measurements for diverse lakes and rivers (Borg 1995; Hart and Hines 1995). Concentrations in dissolved exposure experiments ranged from 0.009 to 1  $\mu\text{g L}^{-1}$  for Ag, 0.18 to 5.6  $\mu\text{g L}^{-1}$  for Cd, 0.04 to 4.7  $\mu\text{g}$

L<sup>-1</sup> for Cr(III), 0.1 to 10 µg L<sup>-1</sup> for Cr(VI) (as Na<sub>2</sub>CrO<sub>4</sub>), and 0.5 to 54 µg L<sup>-1</sup> for Se. For <sup>51</sup>Cr, two redox states, +3 and +6, were examined independently, as these are both common in natural waters and have distinctly different environmental behaviors. Thus, mussels were exposed to ranges of dissolved <sup>51</sup>Cr(III) or <sup>51</sup>Cr(VI), as described elsewhere (Wang et al. 1997). The DOC concentration in this water was 3.7 ± 0.1 mg L<sup>-1</sup>. Stable metal and radioisotope additions were made 24 h prior to mussel exposures to attempt to reach equilibration between different pools (Ma et al. 1999), although speciation of the stable and radioisotopes was not checked and may not have completely equilibrated (Piro et al. 1973).

Hg absorption was measured at one concentration only (42 ng L<sup>-1</sup>) with no stable metal addition and equilibration of radioisotope in water for 15 h. Eight mussels were placed in 600 ml and exposed for 2 h. Absorption efficiency was calculated as <sup>203</sup>Hg atoms absorbed by soft tissues divided by total atoms pumped (using published filtration rates for mussel sizes used; Kryger and Riisgard 1988). The DOC concentration in this experiment was 3.3 ± 0.1 mg L<sup>-1</sup>.

During exposures, water was stirred every 15 min and 2-ml aliquots were removed every 40 min and radioassayed to monitor metal concentrations. Uptake by mussels lowered dissolved metal concentrations by up to 20% over the 2-h exposures. Any feces produced from food ingested prior to experimentation were removed. Mussels were then rinsed, dissected, and radioassayed to determine soft parts and shell activities. Soft parts were then dried (70°C for 48 h) and weighed. Filtration rates were estimated from soft parts dry weight (Kryger and Riisgard 1988); these filtration rates are directly comparable with independent observations (Roditi et al. 1996) for a range of particle concentrations and particle types. Influx rates are expressed as µg metal accumulated g<sup>-1</sup> soft parts d<sup>-1</sup>.

*Trace element efflux rates*—To determine whether efflux rates were influenced by exposure pathway, one group of mussels was exposed to radiolabeled food (*T. pseudonana*) for 6 d, and another was exposed to dissolved trace elements for 7 d. Loss rates were then monitored for 30 d. Phytoplankton was radiolabeled simultaneously with <sup>110m</sup>Ag + <sup>109</sup>Cd + <sup>51</sup>Cr + <sup>75</sup>Se in WC medium, with radioisotope additions corresponding to 7.4 kBq L<sup>-1</sup> (1 nM) of <sup>110m</sup>Ag, 13 kBq L<sup>-1</sup> (2.5 nM) of <sup>109</sup>Cd, 13 kBq L<sup>-1</sup> (2.4 nM) of <sup>75</sup>Se, and 126 kBq L<sup>-1</sup> (0.3 nM) of <sup>51</sup>Cr.

For food exposures, uniformly radiolabeled cells were fed to mussels in beakers containing seven mussels in 500 ml of 0.2-µm filtered Hudson River water. Labeled cells were added every 30 min for 4 h, maintaining an algal concentration of 4.5 × 10<sup>5</sup> cells ml<sup>-1</sup>. Mussels were then rinsed, gamma-counted, and placed in unlabeled water where they were fed unlabeled *T. pseudonana* cells (4.5 × 10<sup>5</sup> cells ml<sup>-1</sup>) to purge their guts of radiolabeled material; the procedure was repeated the following day, for a total of 6 d. After 6 d, mussels were gamma-counted and depurated as described above. After 17 h of depuration, mussels were gamma-counted and two individuals were dissected to determine the partitioning of radioactivity between shell and soft parts in the animals. The live mussels and dissected

shells were depurated for 30 d, during which their radioactivity was monitored. For Hg depuration, mussels were pulse-fed for 30 min as described above and depurated for 26 d.

For dissolved exposures, 12 mussels were placed in 1 liter of 0.2-µm filtered Hudson River water with <sup>110m</sup>Ag, <sup>109</sup>Cd, <sup>51</sup>Cr, and <sup>75</sup>Se in chemical forms described above, except <sup>51</sup>Cr, which was added as Cr(VI) (Na<sub>2</sub>CrO<sub>4</sub> in distilled water). Additions for day 1 exposures were 0.3 kBq L<sup>-1</sup> (0.04 nM) of <sup>110m</sup>Ag, 0.4 kBq L<sup>-1</sup> (0.08 nM) of <sup>109</sup>Cd, 15 kBq L<sup>-1</sup> (2.8 nM) of <sup>75</sup>Se, and 19 kBq L<sup>-1</sup> (0.3 nM) of <sup>51</sup>Cr. Mussels were exposed for 8 h, gamma-counted, and placed in unlabeled 0.2-µm filtered Hudson River water with unlabeled *T. pseudonana* (4.5 × 10<sup>5</sup> cells ml<sup>-1</sup>) for 12 h when the procedure was repeated, for a total of 7 d. During days 2–7, concentrations of <sup>109</sup>Cd, <sup>75</sup>Se, and <sup>51</sup>Cr were lowered and <sup>110m</sup>Ag increased to 0.7 kBq L<sup>-1</sup> (0.1 nM), 0.3 kBq L<sup>-1</sup> (0.06 nM) for <sup>109</sup>Cd, 4 kBq L<sup>-1</sup> (0.7 nM) for <sup>75</sup>Se, and 14 kBq L<sup>-1</sup> (0.2 nM) for <sup>51</sup>Cr (changes were made to produce animals with radioactivity just sufficient to count accurately). After 7 d of exposure, mussels were rinsed and gamma-counted, and three mussels were dissected; live mussels and shells were then depurated for 30 d as described above. No measurements were made of <sup>203</sup>Hg efflux from mussels following uptake from the dissolved phase.

*Radioactivity measurements*—The gamma radioactivity of the samples was counted with intercalibrated gamma detectors equipped with well-type NaI(Tl) crystals. Spillover of the emissions of one radioisotope into another's counting window was corrected, and counts were related to standards for all isotopes and all assays; counts were corrected for background. Gamma emissions of <sup>110m</sup>Ag were measured at 658 keV, <sup>109</sup>Cd at 88 keV, <sup>203</sup>Hg at 279 keV, <sup>75</sup>Se at 264 keV, and <sup>51</sup>Cr at 320 keV. Counting times were adjusted to yield propagated counting errors generally of <5%. <sup>14</sup>C samples were counted with a Packard Tri-Carb 2100-TR liquid scintillation analyzer.

## Results

*Particle concentration factors*—All particle types (foods) concentrated all of the radioisotopes to which they were exposed. Concentration factors on a volume basis (VCF) and dry weight basis (DCF) varied by over 2 orders of magnitude when comparing different elements concentrated on one algal species or when comparing uptake of one element onto different particle types (Table 1). Generally Cr was concentrated most (DCFs ranging from 1 to 19 × 10<sup>4</sup>), followed by Ag, Hg, and Cd. Se was least concentrated (DCFs ranging from 2.1 × 10<sup>2</sup> to 5.4 × 10<sup>4</sup>). The lowest DCFs generally were for bacteria. Because the bacterial particle treatment used a natural assemblage, there was a potential for selection of metal tolerance among the resident bacteria, and the different growth rates and coloration patterns in each culture suggest that this did occur. Bacterial cells in the dual labeled <sup>14</sup>C + <sup>51</sup>Cr culture were large and grew quickly (6 d) to a milky-white color (density = 6.0 × 10<sup>8</sup> cells ml<sup>-1</sup>, mean cell volume = 0.6 µm<sup>3</sup>, Table 1), while those in the <sup>110m</sup>Ag + <sup>109</sup>Cd + <sup>75</sup>Se culture were smaller and grew slowly (12 d)

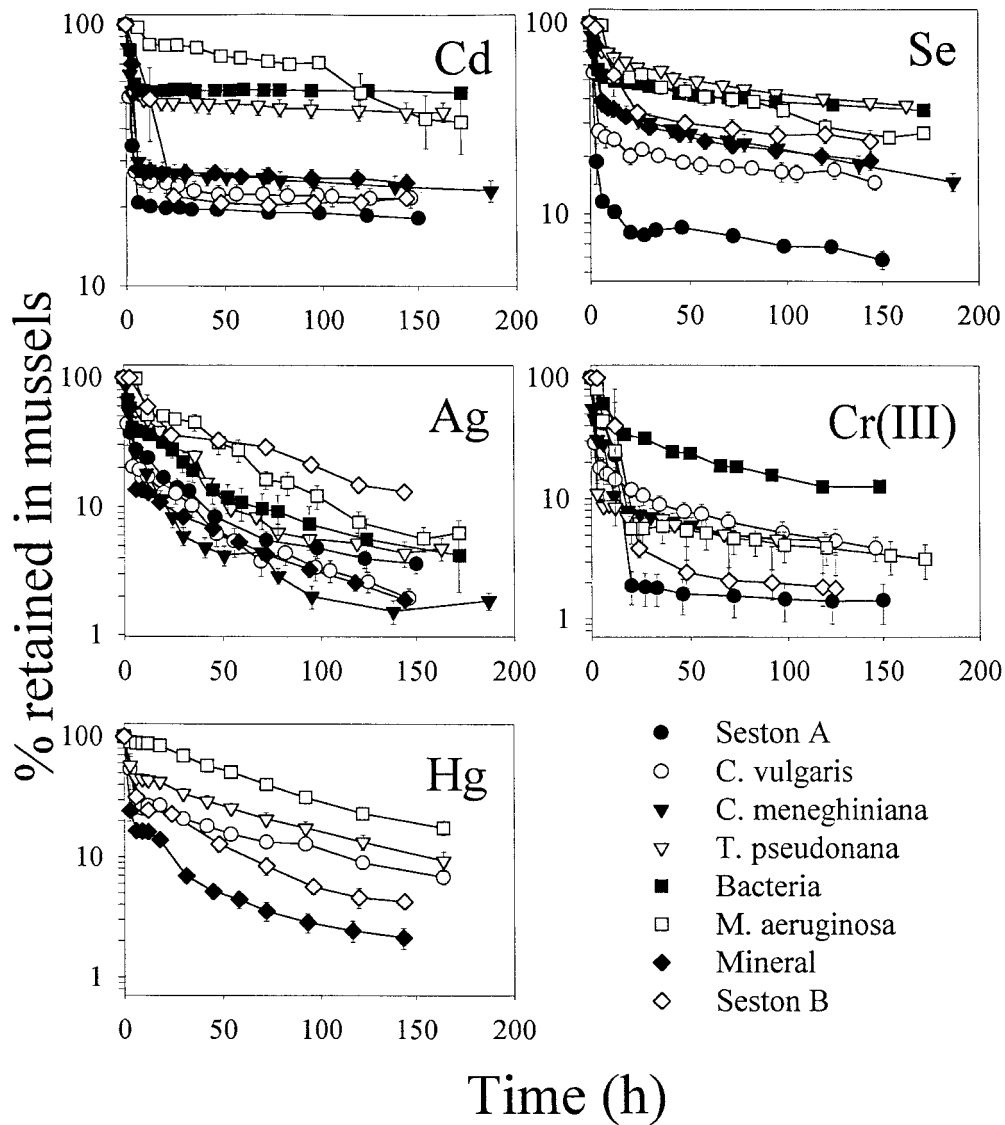


Fig. 2. Percentage of ingested radioisotope retained during depositions following 30 min pulse feedings on uniformly labeled food particles. In each experiment a single particle type was fed to the mussels. Points are means  $\pm$  1 SE;  $n = 5-7$  mussels.

to a pink color (density =  $3.0 \times 10^8$  cells  $\text{ml}^{-1}$ , mean cell volume =  $0.2 \mu\text{m}^3$ ).

*Trace element and C assimilation from food in D. polymorpha*—The retention of trace elements in whole animals after feeding on different particle types is shown in Fig. 2. In general, ingested elements are rapidly lost by defecation in the first 24 h, after which there is slower loss, but the pattern of defecation over time differed between elements. Unassimilated Cd and Cr, for example, were defecated in the first 24 h, and often even in the first 12 h, after which there was very little radioisotope in feces (Fig. 1). Unassimilated Ag, Hg, and Se, by contrast, were defecated in two peaks occurring within the first 30 h (Fig. 1).

Assimilation efficiencies (AEs) of trace elements were calculated as percentage of ingested radioisotope retained after 72-h depuration (Table 2). These values do not differ

appreciably from AEs calculated using the y intercepts of the slowly exchanging pools because the loss rates were relatively small. AEs varied by more than an order of magnitude between trace elements and between food types. Trace element AEs were of the following order: Cd > Se > Hg > Ag  $\geq$  Cr. Elements were generally least assimilated from the seston assemblages and most assimilated from *M. aeruginosa*, *T. pseudonana*, and bacteria assemblages. C AE (Table 2) was highest from the cyanophyte *M. aeruginosa* (85%) and lowest from the chlorophyte *C. vulgaris* (42%).

Gut passage times (GPTs) were variable among particle types and trace elements (Table 3). For a specific food, GPTs varied by up to an order of magnitude depending on the element, and for a specific element, GPTs varied by >1 order of magnitude between food types (Table 3). When comparing GPTs of an element ingested in different foods, longer

Table 3. Gut passage times (time for 90% egestion) of ingested trace elements from eight foods. Values given as means  $\pm$  1 SE;  $n = 5$  for all treatments except bacteria  $^{51}\text{Cr}$  treatment, for which  $n = 7$ . Also shown are percentages of overall digestion corresponding to intracellular digestion for each particle type (means  $\pm$  1 SE;  $n = 5$ ). ND, not determined.

Particle type	Gut passage time (h)					Digestive partitioning (% intracellular)
	Ag	Cd	Cr	Hg	Se	
<i>T. pseudonana</i>	28 $\pm$ 2	4.7 $\pm$ 1.7	3.0 $\pm$ 0.6	53 $\pm$ 5	32 $\pm$ 4	25 $\pm$ 3
<i>C. meneghiniana</i>	23 $\pm$ 4	3.9 $\pm$ 0.7	11 $\pm$ 2	ND	20 $\pm$ 3	15 $\pm$ 1
<i>C. vulgaris</i>	7.6 $\pm$ 2.7	4.0 $\pm$ 1.0	7.4 $\pm$ 3.7	32 $\pm$ 8	4.4 $\pm$ 1.1	8.2 $\pm$ 1.3
<i>M. aeruginosa</i>	55 $\pm$ 6	50 $\pm$ 7	17 $\pm$ 6	78 $\pm$ 9	99 $\pm$ 6	40 $\pm$ 4
Bacteria assemblage	25 $\pm$ 2	10 $\pm$ 2	46 $\pm$ 5	ND	24 $\pm$ 3	27 $\pm$ 2
Seston assemblage (A)	17 $\pm$ 2	4.2 $\pm$ 0.4	8.2 $\pm$ 3.3	ND	3.7 $\pm$ 0.5	12 $\pm$ 1
Seston assemblage (B)	6.0 $\pm$ 0.3	5.4 $\pm$ 0.2	ND	21 $\pm$ 2	15 $\pm$ 3	7.3 $\pm$ 0.7
Mineral assemblage	22 $\pm$ 6	ND	14 $\pm$ 2	32 $\pm$ 4	30 $\pm$ 7	7.6 $\pm$ 2.4

GPTs appear to coincide with higher AEs for that element (Fig. 3); however, when comparing GPTs for all elements for one food type, longer GPTs do not predict higher AE. For example, Cd often has the shortest GPT and the highest AE, and Ag has one of the longest GPTs but often the lowest AE (Table 3).

Ag was used as a tracer of digestive partitioning (intracellular versus extracellular digestion) of food, because Ag is processed in the digestive gland, as indicated by its biphasic egestion pattern (Fig. 1), and because it is not highly assimilated. In fact, its AE was comparable to that of Cr, which is widely used as an inert tracer (e.g., Calow and Fletcher 1972). Although this method has been used in previous work with *M. edulis* (Wang et al. 1995), it is used cautiously here as an index of digestive partitioning because, although it is poorly assimilated, Ag is not fully inert to digestion, and because metal and organic material may follow different pathways to some degree in the gut. The percentage of intracellular digestion was calculated by monitoring  $^{110\text{m}}\text{Ag}$  in feces as:

$$\text{Percentage intracellular} = (A_{\text{feces}>12\text{h}}/[A_{\text{total feces}}]) \times 100 \quad (3)$$

where  $A_{\text{feces}>12\text{h}}$  is the radioactivity of  $^{110\text{m}}\text{Ag}$  in feces between 12 h and the end of depuration, and  $A_{\text{total feces}}$  is the radioactivity of  $^{110\text{m}}\text{Ag}$  in feces produced throughout the depuration period. The intracellular digestion ranged from about 7% for seston assemblage A to 40% for *M. aeruginosa* (Table 3). Generally, AEs for C, Ag, Cd, Hg, and Se (but not Cr) were higher for food types that underwent more intracellular digestion, such as *M. aeruginosa* and the bacteria assemblage (Fig. 4). Bacteria were excluded from correlations for Cr and C in Fig. 4 because intracellular digestion data and Cr and C AEs were derived from independent cultures with different cellular characteristics. The nonlinear pattern observed for C and Se (Fig. 4) may indicate that, for elements with high AEs, a maximum AE exists that does not increase with further increases in intracellular digestion.

Overall, C AEs were weakly correlated with trace element AEs (Fig. 5). Bacteria were excluded from correlations for Ag, Cd, and Se (Fig. 5) because these AEs were derived

from a culture that may not be comparable to the culture used to determine C AE and Cr AE.

*Filtration rates of suspended particles*—FRs were calculated for some particle treatments (Table 4) and compared with rates predicted by the equations of Kryger and Riisgard (1988) for animals of comparable shell lengths. Actual FRs are close to those predicted for all particles except bacteria, which were cleared at only 35% of clearance rates predicted for larger particles.

*Release of trace elements from mussel feces*—Release of metals from fecal pellets was characterized by 2 to 3 compartments of constant slope (Fig. 6), reflecting slower loss over time. Generally, Cd and Hg were released most rapidly ( $tr_{1/2}$  values ranged between 1.6 and 14 d) and Ag was released most slowly ( $tr_{1/2}$  values ranged between 12 and 99 d) (Table 5). Cr loss was examined for mineral assemblage feces only; from its  $tr_{1/2}$  value of  $59 \pm 37$  d, it appears to be released more slowly than Ag (Table 5).

*Trace element influx rates from the dissolved phase*—Influx rates from the dissolved phase were significantly related to dissolved concentration for all elements (see  $r^2$  values in Table 6). Regression slopes of log influx rate versus log trace element concentration were close to 1, with Cr(III) having the lowest slope, about 0.75 (Table 6). The influx rate can be interpreted as the product of an uptake rate constant ( $K_u$ ) and trace element concentration in water ( $C_w$ ); the uptake rate constant ( $K_u$ ) can in turn be understood as the product of an absorption efficiency and the filtration rate (FR).  $K_u$  was determined from the y intercept of the regression equation of log-transformed influx rate versus dissolved concentration using the mean water concentration over the 2-h exposure period. The absorption efficiency, defined as the percentage of atoms pumped by the mussel that are absorbed, can be determined if FRs are known. FRs were not directly measured in the solute uptake experiments; however, dry weights of soft parts were measured and used with FR equations of Kryger and Riisgard (1988)

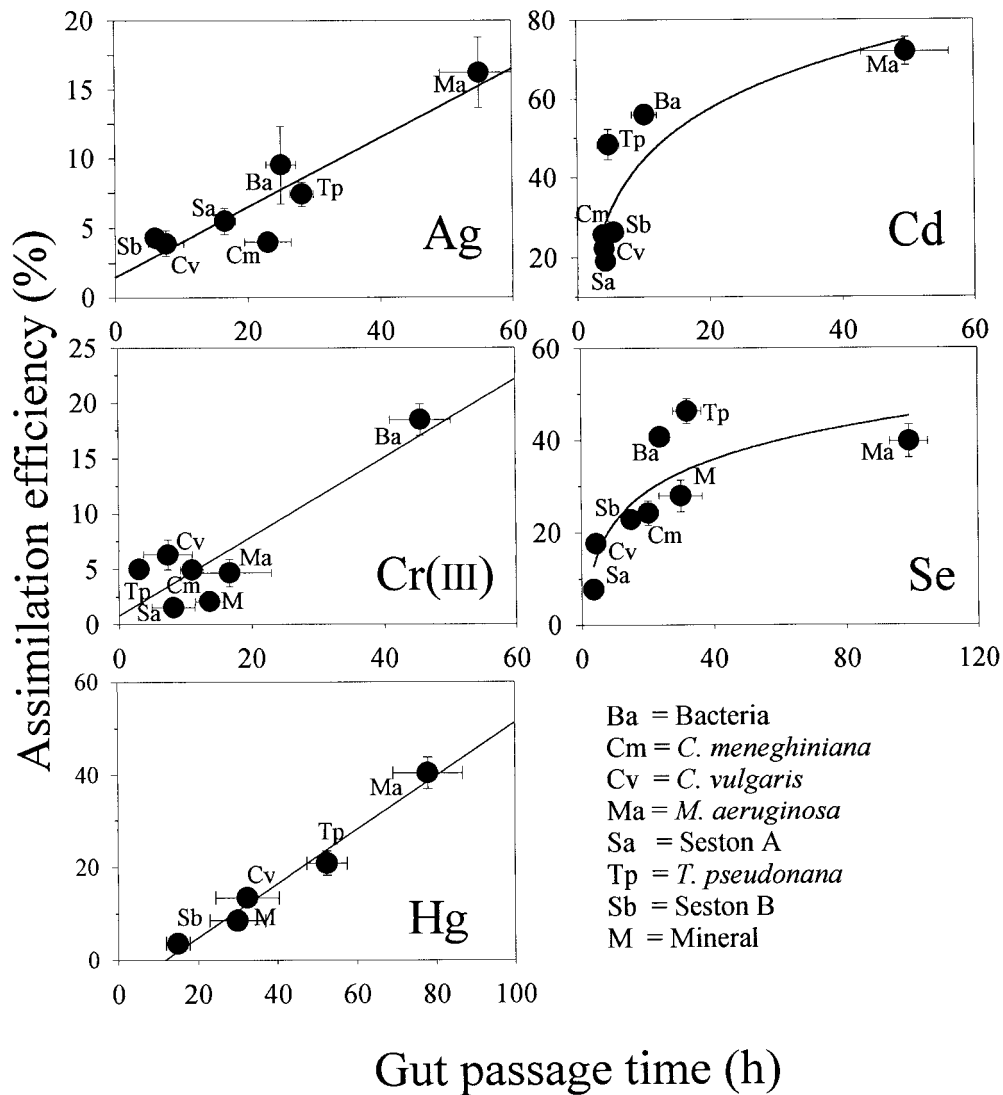


Fig. 3. Elemental assimilation efficiencies as a function of gut passage times for elements, after ingestion of seven to eight particle types. Silver (Ag),  $y = 0.25x + 1.5$ ,  $P < 0.01$ ,  $r^2 = 0.85$ ; cadmium (Cd),  $y = 458x^{0.037} - 454$ ,  $r^2 = 0.75$ ; selenium (Se),  $y = 264x^{0.034} - 263$ ,  $r^2 = 0.67$ ; chromium(III) (Cr[III]),  $y = 0.36x + 0.8$ ,  $P < 0.01$ ,  $r^2 = 0.78$ ; mercury (Hg),  $y = 0.58x - 6.9$ ,  $P < 0.01$ ,  $r^2 = 0.97$ . Points are means  $\pm$  1 SE;  $n = 5-7$  mussels.

to estimate FRs for individual mussels. Estimated FRs generally ranged from 266 to 298 L g<sup>-1</sup> dry tissue weight d<sup>-1</sup>. Resulting absorption efficiencies (Table 6) ranged from 0.03% for Se to 1.87% for Ag. Absorption efficiencies were in the following order: Ag > Hg > Cd > Cr(III) > Cr(VI) > Se.

**Trace element efflux rates**—Efflux rates following 6-d radiolabeled food exposures and 7-d dissolved exposures declined over time, reflecting greater proportional loss from the slowly exchanging compartments within the mussel (Fig. 7). Depuration data sets were grouped into sections of constant slope by visually inspecting data, and the slowest efflux (the last compartment) was interpreted as the physiological loss rate for all elements except Ag. Silver efflux was determined from an earlier compartment (6 to 12 d for food, 1.4 to 7.4

d for dissolved exposures), because whole animal efflux rates at time points later in the depuration probably reflected efflux from the shell instead of soft parts because a greater proportion of the total body burden was on the shell at that time (Table 7).

Efflux rate constants (from the slowest exchanging pools) were lowest for Cd (0.012 and 0.011 d<sup>-1</sup> for food and water treatments, respectively) and highest for Ag (0.067 and 0.084 d<sup>-1</sup> for food and water) (Table 8). The source of exposure—food or water—had a significant ( $P < 0.05$ ) but minor effect on efflux rates for Cr and Se and did not significantly affect efflux rates of Ag and Cd. Biological half-lives for Cd, Cr, and Se (between ~20 and 65 d) are similar to those observed for *M. edulis* (Wang et al. 1996), but Ag is lost more rapidly in *D. polymorpha* (8 to 10 d versus 22 to 38 d for *M. edulis*).

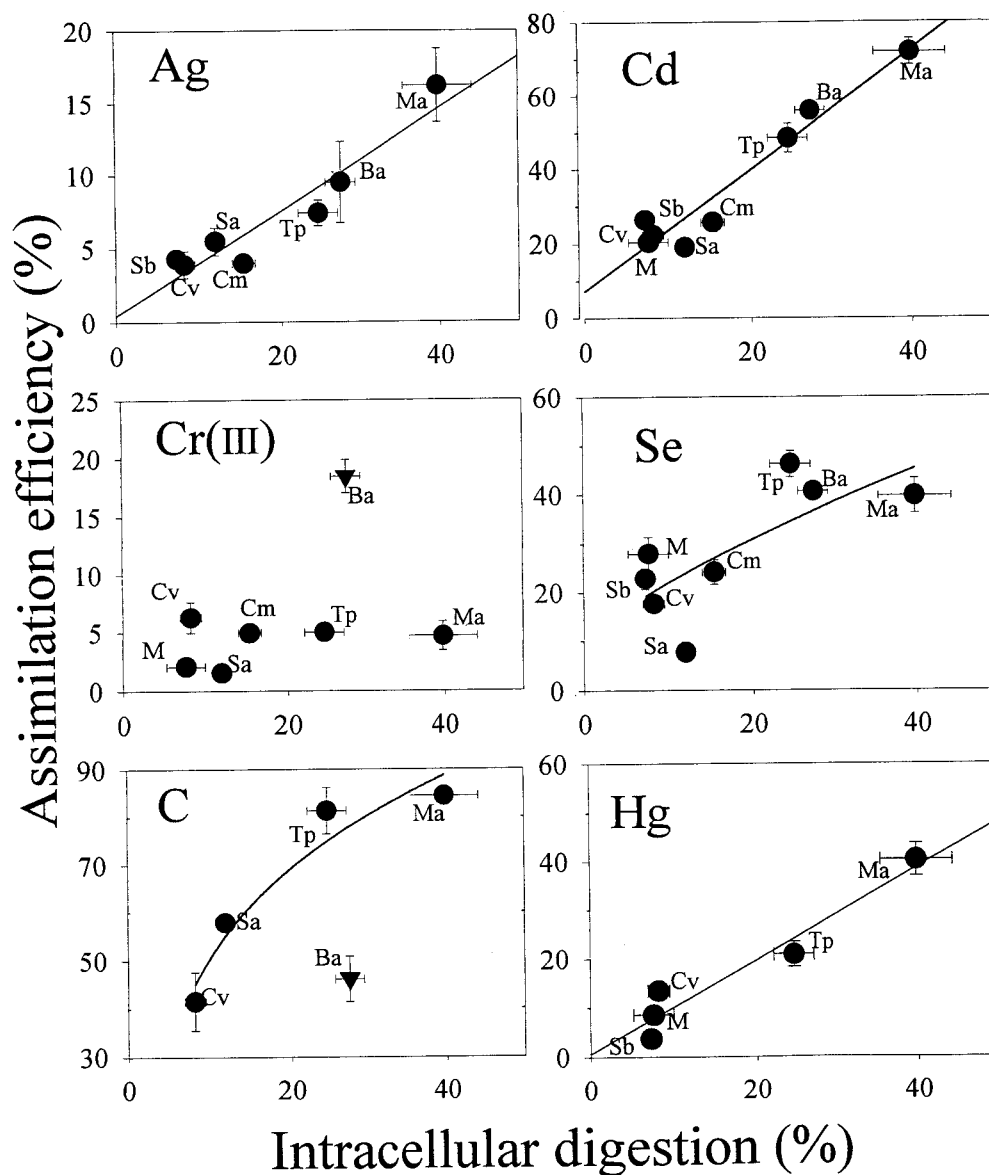


Fig. 4. Relationship of metal assimilation efficiencies with food digestive partitioning (intracellular digestion), after ingestion of seven to eight particle types. Symbols as defined in Fig. 3. Silver (Ag),  $y = 0.35x + 0.5$ ,  $P < 0.01$ ,  $r^2 = 0.90$ ; cadmium (Cd),  $y = 1.6x + 7.2$ ,  $P < 0.001$ ,  $r^2 = 0.94$ ; selenium (Se),  $y = 2.8x^{0.70} + 8.0$ ,  $r^2 = 0.54$ ; carbon (C),  $y = 446x^{0.05} - 454$ ,  $r^2 = 0.94$ ; mercury (Hg),  $y = 0.93x + 0.6$ ,  $P < 0.01$ ,  $r^2 = 0.93$ . The bacteria assemblage (Ba) was omitted from regression analysis for carbon and chromium because the assimilation efficiencies and the intracellular digestion of these elements were measured on independent cultures, which in the bacterial treatment contained different strains (see text). Points are means  $\pm$  1 SE;  $n = 5-7$  mussels.

## Discussion

**Particle concentration factors**—Concentration factors are an index of the reactivity of the metals for different particle types. Metals that do not get concentrated at all by particles (either by active uptake or passive adsorption) can only be accumulated in mussels from the dissolved phase. Concentration factors are affected by metal speciation in water, which is a function of complexation with dissolved organic matter and inorganic ligands such as chloride and sulfide.

Concentration factors for some metals decrease with salinity due to chlorocomplexation and the presence of competing ions (Fisher and Reinfelder 1995); consequently, concentration factors for these metals are expected to be somewhat higher in low-DOC fresh water than in coastal surface seawater. Comparisons of algal concentration factors of metals with marine algae of the same taxonomic grouping indicate that Cd, which is strongly chlorocomplexed (Turner et al. 1981), was concentrated by phytoplankton about an order of magnitude more in this study than in marine systems (Fisher

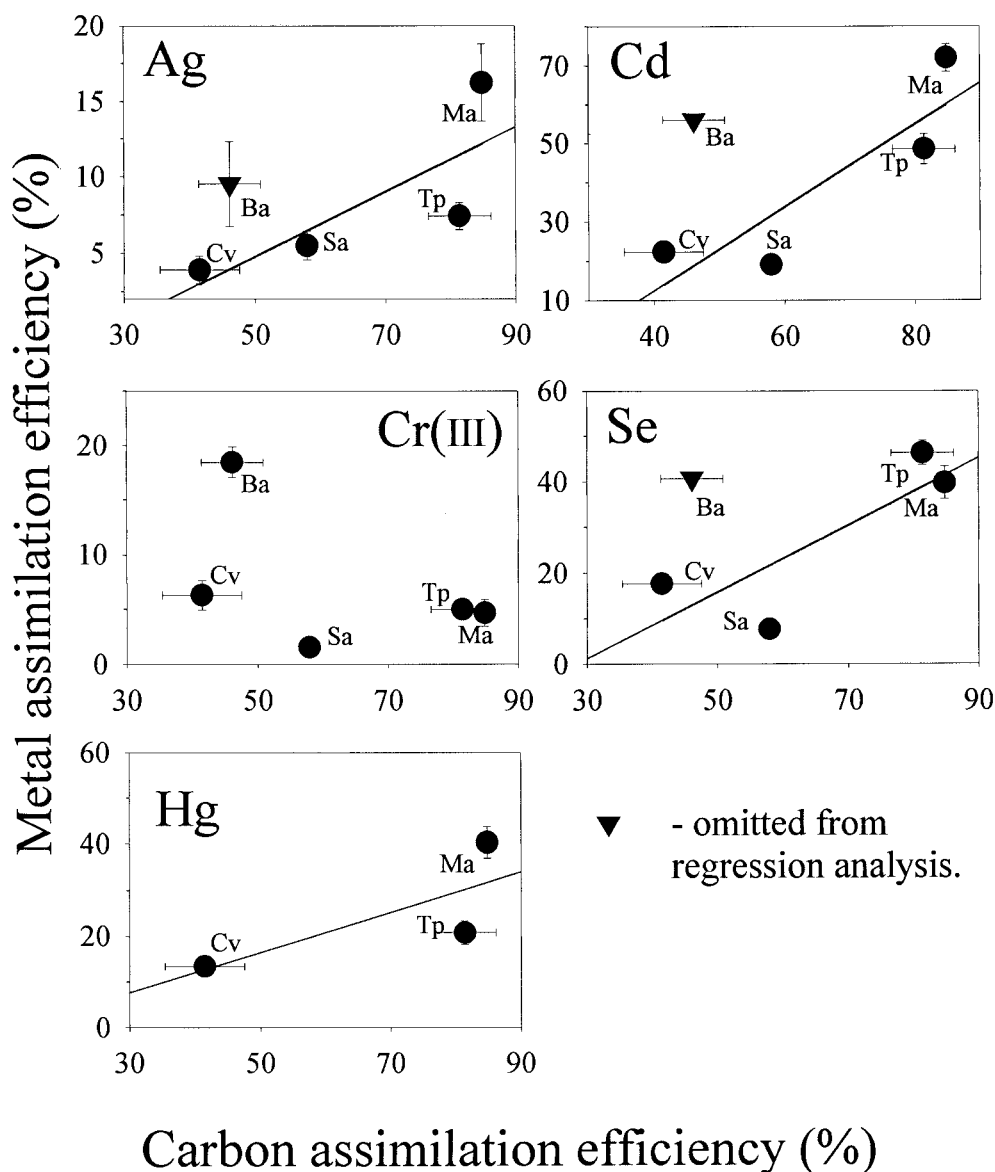


Fig. 5. Relationship of metal assimilation efficiencies with C assimilation, after ingestion of five particle types. Silver (Ag),  $y = 0.21x - 5.8$ ,  $r^2 = 0.62$ ; cadmium (Cd),  $y = 1.1x - 30$ ,  $r^2 = 0.77$ ; selenium (Se),  $y = 0.73x - 21$ ,  $r^2 = 0.68$ ; mercury (Hg),  $y = 0.4x + 5.6$ ,  $r^2 = 0.58$ ; none of the regressions were significant ( $P > 0.05$ ). The bacteria assemblage (Ba) was omitted from regression analysis for Ag, Cd, and Se because Ag, Cd, and Se assimilation and C assimilation were measured on independent cultures, which in the bacterial treatment contained different strains (see text). Symbols as defined in Fig. 3.

and Reinfelder 1995). The other metals did not show as large a discrepancy between marine and freshwater algae.

In comparing our results with other studies in fresh waters, we can summarize as follows. For Ag, our concentration factors were at the low end of the range observed for suspended particles (Santschi et al. 1986; Garnier et al. 1997). For Cd, our mean dry weight concentration factor for all particle types ( $4.8 \times 10^4$ ) was at the upper end of the range observed in some freshwater environments ( $1.6\text{--}3.0 \times 10^4$ ) (Sigg 1985; Garnier et al. 1997) but below values in some Swiss lakes ( $0.5\text{--}5 \times 10^5$ ) (Sigg 1987) and other freshwater

environments ( $1.1\text{--}4.5 \times 10^5$ ) (Radovanovic and Koelmans 1998). Santschi et al. (1986) obtained partition coefficients (Kd values) for Se and Cr(III) of  $1 \times 10^5$  and  $4 \times 10^5$ , respectively, which are about an order of magnitude higher than our values for these elements. Differences may be due to different complexation by dissolved organic matter in the different waters studied and to variations in particle type, which can also influence concentration factors (e.g., Table 1). The particle reactivity of Hg (concentration factor in natural seston of  $1.1 \times 10^5$ ) was comparable to results from marine studies (Fisher and Reinfelder 1995; Gagnon and

Table 4. Filtration rates (FRs, ml h<sup>-1</sup>) estimated for several particle types and comparison with reported FRs (Kryger and Riisgard 1988); n = 5 except for bacteria, for which n = 9.

Particle	Shell length (mm)	Measured FR	Reported FR	Measured FR divided by reported FR (%)
<i>T. pseudonana</i>	18.0	150±25	187	80
<i>M. aeruginosa</i>	19.0	216±42	210	103
Bacteria assemblage (<1 μm)	22.2	102±12	292	35
Seston assemblage	19.0	241±13	210	115

Fisher 1997) and somewhat lower than a mean value reported for lakes (1.8 × 10<sup>5</sup>) (Watras et al. 1995). For the largely inorganic assemblages (seston A and B and mineral), the general order of particle reactivity was Cr > Ag > Hg > Cd > Se.

Metal assimilation efficiencies in aquatic herbivores generally correlate with the cytoplasmic distribution of metals in algal food (Reinfelder and Fisher 1994; Wang and Fisher 1996). Because the experimental conditions under which the algae are exposed to metals may influence the cytoplasmic distribution of the metals in the algae, metal assimilation in animals may also be affected. As there are scant data on the cellular distributions of metals in freshwater phytoplankton assemblages, we assume here that the fractionation of metals in the algae used in our study are comparable with those in

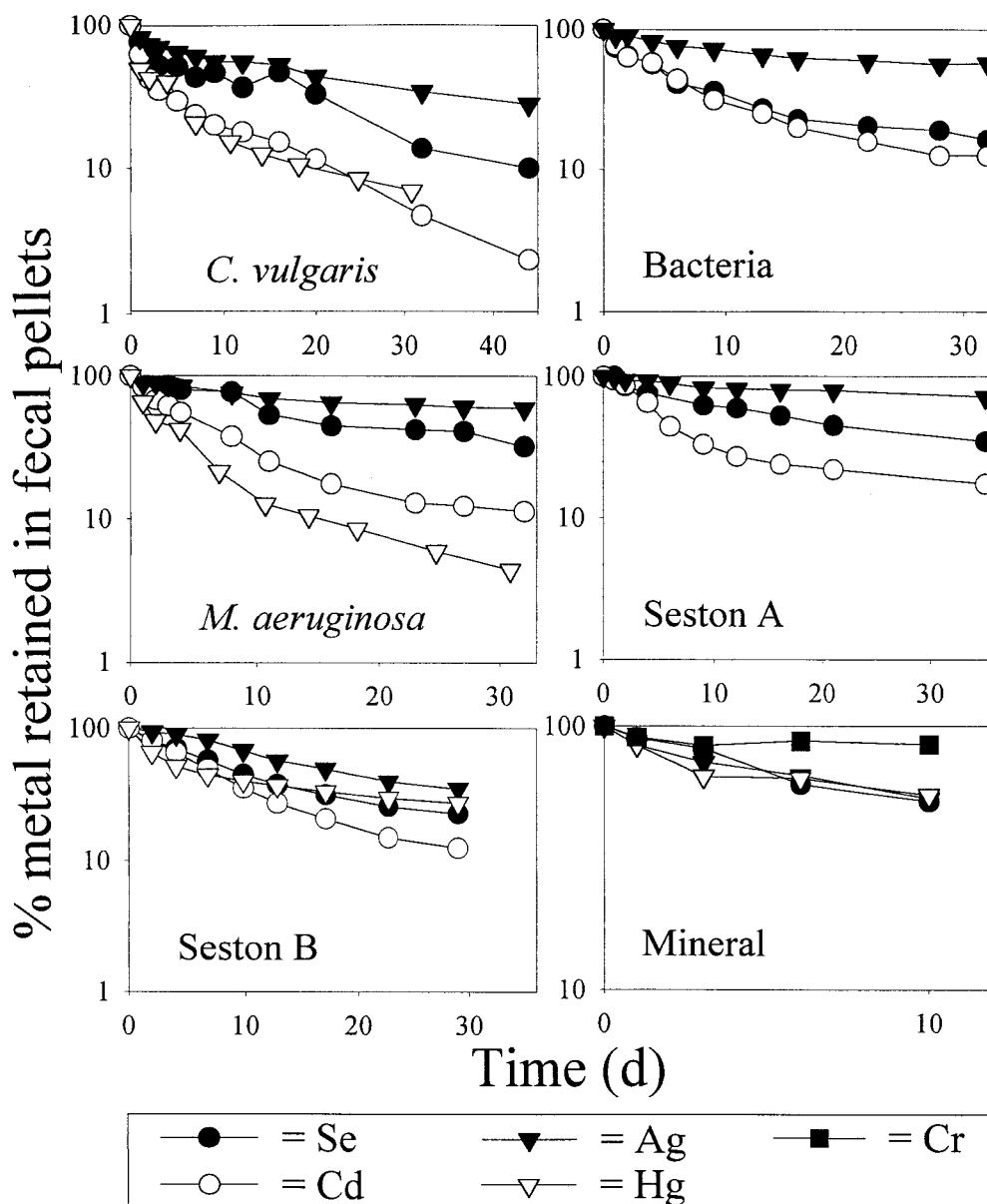


Fig. 6. Retention of metals in fecal pellets incubated in filtered Hudson River water. Loss rates were calculated from compartments of constant slope.

Table 5. Overall retention half-times ( $t_{1/2}$ ) of trace elements in *D. polymorpha* feces. Fecal pellets were produced following feeding on different particle suspensions. Values given as means  $\pm$  1 SE. ND, not determined.

Food	Retention half-time ( $t_{1/2}$ )				
	Ag	Cd	Hg	Se	Cr
<i>C. vulgaris</i>	19 $\pm$ 1	1.6 $\pm$ 0.1	3.7 $\pm$ 1.0	5.0 $\pm$ 0.4	ND
<i>M. aeruginosa</i>	55 $\pm$ 15	5.9 $\pm$ 0.3	3.8 $\pm$ 0.4	17 $\pm$ 6	ND
Bacteria assemblage	42 $\pm$ 9	5.8 $\pm$ 0.3	ND	6.1 $\pm$ 0.4	ND
Seston assemblage A	99 $\pm$ 9	5.3 $\pm$ 0.3	ND	18 $\pm$ 2	ND
Seston assemblage B	18 $\pm$ 1	6.7 $\pm$ 0.1	5.6 $\pm$ 0.8	9.1 $\pm$ 0.3	ND
Mineral assemblage	12 $\pm$ 1	ND	14 $\pm$ 4	10 $\pm$ 1	59 $\pm$ 37
Average	41 $\pm$ 13	5.1 $\pm$ 0.9	6.7 $\pm$ 2.3	11 $\pm$ 2	—

natural waters, particularly because we allowed the cells to equilibrate with the metals over several cell division cycles and used metal concentrations that do not greatly exceed natural concentrations. The comparability noted above of the metal concentration factors in the experimental phytoplankton with those in natural waters further suggests that the conditions used in our experiments produced cells representative of those in natural assemblages.

*Trace element and C assimilation from food in D. polymorpha*—The feeding experiments showed that food can be an important source of trace elements to zebra mussels and that food type is an important variable determining bioavailability. Carbon AEs were generally comparable to results with marine mussels; C AE for *M. edulis* fed *T. pseudonana* was 85%, compared to 81% for zebra mussels fed the same diet (Table 9). As with *M. edulis* (Wang and Fisher 1996), the lowest C AEs in zebra mussels were for green algae (Table 2). The low chlorophyte C AE is likely due to refractory cell walls that resist digestion. The C AE from bacteria (46%, Table 2) was higher than the results of Silverman et al. (1995) would suggest. They found that conversion of bacterial-labeled amino acids into zebra mussel protein was 29%. For the clams *Macoma balthica* and *Potamocorbula amurensis*, Decho and Luoma (1991) obtained higher C AE values from bacteria than this study—68 and 75%, respectively. For the mussels *Geukensia demissa* and *M. edulis*, Kreeger and Newell (1996) measured C AEs of 42 and 21%, respectively, from bacterial diets.

The biphasic digestion observed in this study has been

observed in marine bivalves (*P. amurensis* and *M. balthica*, Decho and Luoma 1991; *M. edulis*, Wang et al. 1995) and has been considered indicative of intracellular and extracellular digestion. Extracellular digestion occurs in the stomach with the aid of crystalline styles that produce digestive enzymes. Following this extracellular stage, some of the finer particles are directed to the digestive diverticula, where specialized cells phagocytize and digest them. These digestive stages have been observed in pulse-chase feeding studies and defined operationally by the times two distinct peaks in fecal production are observed, corresponding to extracellular and intracellular feces (Decho and Luoma 1991). Intracellular digestion has been found to be the more efficient pathway for C and trace element assimilation, and digestion may become increasingly intracellular as ingestion rate decreases (Decho and Luoma 1991; Wang et al. 1995). Zebra mussel digestion rates appear to be somewhat greater than in marine mussels, with extracellular digestion complete within 12 h, compared to 17 h for *M. edulis* (Wang et al. 1995). Carbon and metal AEs in marine mussels increase with increasing intracellular digestion (Wang et al. 1995), and zebra mussel digestion is comparable in these respects; AEs of all elements except Cr increased with increasing intracellular digestion (Fig. 4).

The y intercepts and slopes of regression lines in Figs. 3 and 4 can be used to better understand AEs in each digestive compartment. For example, in the case of Hg, no assimilation occurs when GPT of Hg is under 12 h (Fig. 3). As noted earlier, digestion in the intestine is completed within 12 h, and all digestion afterwards (> 12 h) is in the digestive gland

Table 6. Regression equations based on log-transformed data for metal influx rate vs. dissolved metal concentration,  $k_{\mu}$  values, and absorption efficiencies (%). Values for slopes  $\pm$  1 SE. ND, not determined.

Element	Equation	$r^2$	$k_{\mu}$ range ( $l\ g^{-1}d^{-1}$ )	Absorption efficiency (%)	
				Mean	Range
Ag	$I_{\mu} = 5.062[C_w]^{0.868 \pm 0.050}$	0.990	3.56–7.19	1.87	1.32–2.66
Cd	$I_{\mu} = 2.726[C_w]^{1.085 \pm 0.035}$	0.998	2.30–3.22	1.02	0.90–1.15
Cr(III)	$I_{\mu} = 1.313[C_w]^{0.754 \pm 0.068}$	0.976	0.82–2.10	0.47	0.29–0.75
Cr(VI)	$I_{\mu} = 0.742[C_w]^{1.016 \pm 0.075}$	0.984	0.44–1.24	0.27	0.16–0.46
Hg	ND	ND	1.84–4.75	1.17	0.66–1.79
Se	$I_{\mu} = 0.071[C_w]^{0.935 \pm 0.044}$	0.993	0.05–0.10	0.03	0.02–0.04

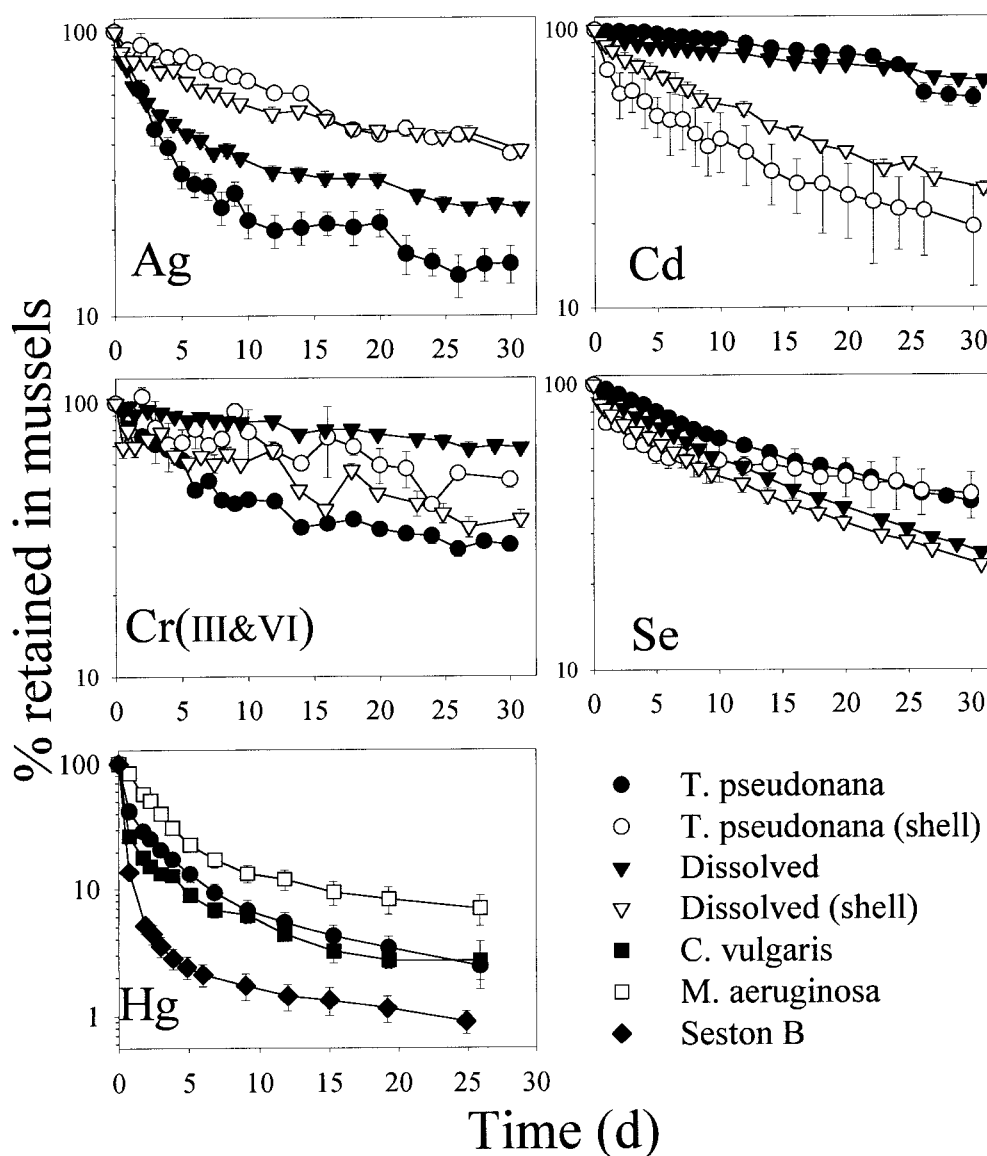


Fig. 7. Retention of radioisotopes in whole mussels and dissected shells following long-term exposures to metals from food (*Thalassiosira pseudonana*) and water. Mercury (Hg) depurations included exposures to *Microcystis aeruginosa*, *Chlorella vulgaris*, and seston assemblage B, but no exposures to the dissolved phase. Exposure duration was 6 d for food and 7 d for dissolved (all Hg exposures were 30 min and no shells were depurated). Points are means  $\pm$  1 SE. For food exposures,  $n = 5$  mussels and 2 shells; for dissolved exposures,  $n = 9$  mussels and 3 shells.

(Fig. 1). Thus, the relationship of Hg AE with Hg GPT (Fig. 3) shows that to be assimilated, an Hg atom must enter the digestive gland. Furthermore, the equation describing the relationship between Hg AE and percentage of intracellular digestion (Fig. 4) has a slope close to 1 (0.96), which suggests very high (possibly 100%) AE of Hg in the digestive gland. The low desorption of Hg from particles into fluids containing pH conditions similar to bivalve guts (Gagnon and Fisher 1997) is consistent with the observation that this metal displays negligible assimilation in the intestine of *D. polymorpha*. The absence of any Cd in digestive gland feces (Fig. 1) is consistent with 100% AE of Cd in the digestive gland. Cd also appears to be assimilated to some extent in

the intestine, as indicated by a positive y intercept (Fig. 4). Perhaps as food quality increases, as indicated by an increasing fraction being sent to the digestive gland, the same food is also a better source of Cd in the intestine, which in turn could account for a slope of  $>1$  (Fig. 4). The slope of the Ag regression line (0.36, Fig. 4) indicates relatively inefficient assimilation of Ag in the digestive gland (36%); our use of Ag as a tracer for estimating digestive partitioning assumes no assimilation of Ag in the digestive gland, and any Ag assimilation in the digestive gland thus produces an underestimate of percentage of intracellular digestion. If percentage intracellular values are increased to compensate for Ag assimilation, all slopes in Fig. 4 decrease; slopes for Hg,

Table 7. Loss of metals from shell (k), retention half-time ( $t_{1/2}$ ), and percentage of radioisotope on shell before and after depuration following long-term uptake from food (*T. pseudonana*) and the dissolved phase. Values given as means  $\pm$  1 SE; % on shell particulate: initial  $n = 2$ , final  $n = 5$ ; % on shell dissolved: initial  $n = 3$ , final  $n = 9$ ; k and  $t_{1/2}$  particulate:  $n = 2$ ; k and  $t_{1/2}$  dissolved:  $n = 3$ .

Element	Treatment	Period (d)	% on shell (initial)	% on shell (final)	k (d <sup>-1</sup> )	$t_{1/2}$ (d)
Cd	Particulate	0–30	1.3 $\pm$ 0	2.9 $\pm$ 0.6	0.048 $\pm$ .009	15 $\pm$ 3
	Dissolved	0–31	30 $\pm$ 1	9.1 $\pm$ 0.8	0.041 $\pm$ .001	17 $\pm$ 1
Cr	Particulate	0–30	4.9 $\pm$ 0.1	7.9 $\pm$ 0.4	0.021 $\pm$ .001	33 $\pm$ 2
	Dissolved	0–31	23 $\pm$ 7	17 $\pm$ 1	0.027 $\pm$ 0	26 $\pm$ 0
Se	Particulate	0–30	3.0 $\pm$ 0.6	6.4 $\pm$ 0.4	0.021 $\pm$ .007	37 $\pm$ 12
	Dissolved	0–31	64 $\pm$ 8	67 $\pm$ 2	0.043 $\pm$ .001	16 $\pm$ 0
Ag	Particulate	0–30	39 $\pm$ 1	84 $\pm$ 4	0.033 $\pm$ .001	21 $\pm$ 0
	Dissolved	0–31	53 $\pm$ 4	86 $\pm$ 1	0.027 $\pm$ .002	26 $\pm$ 1

Ag, and Cd become 0.68, 0.27, and 1.2, respectively, which indicates assimilation in the digestive gland of 68, 27, and 120%. The latter value for Hg is more plausible than 100%, given that digestive gland feces (>12 h, Fig. 1) contain some unassimilated Hg (Fig. 1).

The greater metal AE with increasing GPT for all elements is likely due to more efficient assimilation when an element is retained longer in the digestive tract and the greater amount of intracellular digestion occurring with longer GPT (Decho and Luoma 1991; Wang and Fisher 1996). For *M. edulis*, Ag, Am, C, Cd, Co, and Zn AEs were highly dependent on GPT, with the shortest GPT observed for chlorophytes (Wang and Fisher 1996), similar to results obtained for Hg, Ag, Cd, and Se in *D. polymorpha*. In this study, Cd and Se AEs appear to reach a maximum at about 70 and 50%, respectively.

Patterns of defecation differed among elements. Generally, the unassimilated fraction of Cd and Cr was defecated

within 12 h (i.e., short GPTs for these elements) although exceptions were noted in *M. aeruginosa* and bacteria (Table 3). Hg, Se, and Ag had longer GPTs and were egested over a longer time (up to 3 d). The absence of Cr in feces after 12 h may have been due to its association with a refractory cell wall that passes directly through the digestive tract with no intracellular digestion. Wang and Fisher (1996) found 98% of Cr was associated with the cell wall in four different algal species, including *T. pseudonana*. The high AE of Cr from bacteria, also observed by Decho and Luoma (1991) in two marine clams, may be due to the passage of bacterial-sized particles into the digestive gland; the mean cell volume of the bacteria used in these experiments was <0.64  $\mu\text{m}^3$  (Table 1), which is only 5% of the volume of the next largest particle type investigated (*C. vulgaris* at 12  $\mu\text{m}^3$ ).

The correlation of metal AE with C AE, albeit a weak one, indicates that these metals are associated with bioavailable organic matter in the algal cells and may follow similar

Table 8. Efflux rate constants (k) and biological half-lives ( $t_{1/2}$ ) of trace elements in *D. polymorpha*, determined by compartmental analysis of trace element depuration following uptake from diatoms (*T. pseudonana*; means  $\pm$  1 SE;  $n = 5$ ) or dissolved phase (means  $\pm$  1 SE;  $n = 9$ ). Asterisk (\*) indicates that particulate and solute k values are significantly different from each other ( $P < 0.05$ ) when comparing slowly exchanging pools. Hg depuration rates were determined following uptake from food only (four food types shown).

Element	Period (d)	% in compartment	k (d <sup>-1</sup> )	$t_{1/2}$ (d)
Particulate				
Cd ( <i>T. pseudonana</i> )	0–24	100	0.012 $\pm$ .001	60 $\pm$ 3
Cr ( <i>T. pseudonana</i> )	8–30	51	*0.019 $\pm$ .002	37 $\pm$ 4
Se ( <i>T. pseudonana</i> )	10–30	83	*0.026 $\pm$ .001	27 $\pm$ 1
Ag ( <i>T. pseudonana</i> )	6–12	15	0.067 $\pm$ .007	10 $\pm$ 1
Hg ( <i>T. pseudonana</i> )	12–26	10	0.056 $\pm$ .004	13 $\pm$ 1
Hg ( <i>C. vulgaris</i> )	12–26	8	0.054 $\pm$ .009	14 $\pm$ 2
Hg ( <i>M. aeruginosa</i> )	12–26	19	0.046 $\pm$ .006	17 $\pm$ 3
Hg (seston assemblage)	6–25	3	0.043 $\pm$ .003	16 $\pm$ 1
Solute				
Cd	2.4–31	92	0.011 $\pm$ .001	65 $\pm$ 4
Cr	4.4–31	94	*0.011 $\pm$ .002	76 $\pm$ 9
Se	13.8–31	76	*0.035 $\pm$ .001	20 $\pm$ 1
Ag	1.4–7.4	28	0.084 $\pm$ .005	8.1 $\pm$ 0.5

Table 9. Comparison of assimilation efficiencies (AE), absorption efficiencies, and efflux rate constants (k) between *Dreissena polymorpha* (*D.p.*) and *Mytilus edulis* (*M.e.*) (from Wang and Fisher 1996; Wang et al. 1996). ND, not determined. There are no measurements of these kinetic parameters for Hg in *M. edulis*.

Parameter		Element					
		Ag	C	Cd	Cr(III)	Cr(VI)	Se
AE <i>Chlorella</i> spp. (%)	<i>D.p.</i>	3.9±2.0	42±6	22±2	6.3±1.3	ND	18±1
	<i>M.e.</i>	12±1	19±3	13±2	1.1±0.7	8.4±2.6	48±4
AE <i>T. pseudonana</i> (%)	<i>D.p.</i>	7.4±0.9	81±5	48±4	5.0±0.5	ND	46±3
	<i>M.e.</i>	17±2	86±4	34±5	0.2±0	1.1±0.2	72±2
AE seston assemblage A (%)	<i>D.p.</i>	5.5±0.9	58±1	19±1	1.5±0.5	ND	7.7±0.4
	<i>M.e.</i>	5.4±1.0	ND	20±6	ND	ND	28±2
Absorption efficiency (%)	<i>D.p.</i>	1.9	ND	1.0	0.5	0.3	0.03
	<i>M.e.</i>	1.5	ND	0.3	0.03	0.1	0.03
k (food) (d <sup>-1</sup> )	<i>D.p.</i>	0.067±.007	ND	0.012±.001	0.019±.002	ND	0.026±.001
	<i>M.e.</i>	0.034±.004	ND	0.014±.003	0.010±.003	ND	0.022±.004
k (dissolved) (d <sup>-1</sup> )	<i>D.p.</i>	0.084±.005	ND	0.011±.001	ND	0.011±.002	0.035±.001
	<i>M.e.</i>	0.019±.001	ND	0.011±.002	ND	0.011±.002	0.026±.002

digestive pathways. Wang and Fisher (1996) also found that Se and Cd AEs were correlated with C AE. The distribution of element in the cytoplasm of marine algae has been found to account for differences in AEs of both essential and non-essential elements in marine bivalve larvae (Reinfelder and Fisher 1994). Although adult bivalves have more complex digestion, the degree of penetration of an element into cytoplasm may explain the partitioning of that element between extracellular and intracellular digestion in bivalves (Wang and Fisher 1996).

Se AEs for *D. polymorpha* were lower than those observed for the marine clam *M. balthica* (86% from diatoms; Luoma et al. 1992) and generally lower than observed for *M. edulis* (Table 9). Cd was generally the most highly assimilated trace element and has a high affinity for metallothioneins (Mason and Jenkins 1995). However, Ag is also a sulfur-seeking metal and is highly reactive with sulfur-rich metallothioneins, but Ag is not highly assimilated. This suggests that other processes, such as substitution for Zn, may explain high Cd assimilation. The Hg AEs from seston and mineral particles (4–9%) are comparable to the results of Gagnon and Fisher (1997) of 1–9% in *M. edulis* fed a variety of mineral particles and natural sediment. Methylated forms of Hg typically have higher AEs in invertebrates (Mason et al. 1996; Gagnon and Fisher 1997), although the extent to which this occurs in *D. polymorpha* was not examined in this study.

*Filtration rates of suspended particles*—The filtration rates of *D. polymorpha* were measured to evaluate the importance of different food types as potential sources of metal for these mussels. There was a threefold greater efficiency of particle clearance for phytoplankton than for bacteria by *D. polymorpha*. This result is consistent with the observations of Jorgensen et al. (1984), who showed that particles <1 μm pass through *D. polymorpha* gills in significant numbers, and of Sprung and Rose (1988), who found particle clearance as low as 0.7 μm. Cotner et al. (1995) observed similar filtration by *D. polymorpha* of 0.36-μm and 0.91-μm particles, at 5 to 37% of the phytoplankton clearance rate.

Clearance efficiencies of 35% suggest that bacteria may be a less important source of trace metals than larger particles (including phytoplankton) for this mussel, given comparable assimilation efficiencies. They also suggest that in an ecosystem such as the freshwater portion of the Hudson River, where bacterial production exceeds algal production (Findlay et al. 1991), bacteria may be exploited as a food source for the zebra mussel despite reduced clearance efficiencies. However, work by Findlay et al. (1998) suggests that direct grazing of Hudson River bacteria by zebra mussels is low.

*Release of trace elements from mussel feces*—Zebra mussel biodeposition rates have been studied and are estimated to be high (Klerks et al. 1996; Roditi et al. 1997). Deposition of feces and pseudofeces near mussel beds may influence biogeochemical cycling of elements by altering the physical matrix binding them and increasing their rate of flux to and retention in the sediment surface. The fate of the feces (re-suspension, consumption by benthic fauna) will be important in influencing cycling, as will the retention times of elements in the feces. Longer retention times may increase trace element burial rates and increase the degree of trophic transfer to organisms consuming fecal pellets. Since the fecal pellet retention of Cr > Ag > Se > Hg ≥ Cd, the residence time of Cr and Ag in zebra mussel biodeposits in the vicinity of mussel beds would be expected to exceed that of Se, Hg, and Cd. The greater retention of Cr and Ag may lead to their greater consumption by detritivores or burial in sediment.

For comparison, we are unaware of any other studies that have determined the rates of metal release from fecal pellets from freshwater invertebrates; however, several studies with metal loss from fecal pellets in marine systems have been conducted. Working with the mussel *Mytilus galloprovincialis*, Fisher et al. (1996) found that Ag was released more rapidly than Cd from the slowly exchanging pool ( $tr_{1/2} = 18 \pm 1$  and  $45 \pm 6$  d, respectively); Ag  $tr_{1/2S}$  in our study are considerably longer (31–120 d in the slowly exchanging pool), but Cd  $tr_{1/2S}$  are similar (10–47 d). For copepod fecal pellets, Cd was lost more rapidly into seawater ( $tr_{1/2} = 1.5$  d) than eight other elements, including Ag (range of  $tr_{1/2S} =$

3–26 d) (Fisher et al. 1991). Reinfelder et al. (1993) found that release of both Cd and Se from radiolabeled sediment trap particles (consisting mostly of zooplankton fecal pellets and marine snow) followed a two-compartment loss, with  $t_{r,1/2}$ s for Cd and Se in the rapid compartment being 4.1 and 2.9 d, respectively, and in the slow compartment being 35.8 and 46.3 d, respectively, values similar to those found in our study.

*Trace element influx rates from the dissolved phase*—Uptake of metals by zebra mussels from solution was a function of the metal concentration over the broad range of concentrations used, consistent with other studies involving marine bivalves (Wang et al. 1996). Influx rate is dependent on the dissolved metal concentration, the metal's absorption efficiency (analogous to AE from food), and biological factors like the animal's pumping rate. Absorption efficiency is a measure of bioavailability and makes it possible to compare biota-metal interactions among different metals as well as different animal species. A submaximal pumping rate of an individual mussel during an experiment, owing to the absence of particles, for example, may lead to difficulties in estimating metal absorption efficiency by reducing the delivery of metal atoms to (and hence the metal concentration near) the gill. At the metal concentrations used, the rate-limiting factor is the delivery of metal atoms to the gill rather than the rate of metal transport across the gill, because no saturation occurs and uptake is a linear function (approximately) of concentration. In a study investigating allometric influences on metal accumulation in mussels, absorption efficiency of dissolved metals was shown to be independent of filtration rate among different sized mussels (Wang and Fisher 1997), which suggests that the results presented here are applicable to a range of mussel sizes and that pumping rates probably do not affect absorption efficiencies under the prevailing experimental conditions. Among the elements examined, the ranking of absorption efficiencies ( $\text{Ag} > \text{Hg} > \text{Cd} > \text{Cr(III)} > \text{Cr(VI)} > \text{Se}$ ) was similar to that observed for *M. edulis* (Wang et al. 1996, 1997).

Ag, Hg, and Cd are reactive with proteins and may bind to membrane proteins, which explains their high absorption from solution. Internal S-rich compounds such as metallothioneins may sequester Ag, Hg, and Cd after transport across membranes, as occurs following uptake from food. In seawater,  $\text{AgCl}^0$  is the dominant Ag species, while in freshwater with lower  $\text{Cl}^-$  ion concentrations,  $\text{Ag}^+$  can be dominant (Turner et al. 1981). It is not known whether this would make Ag more or less bioavailable, however. While studies have generally shown that the free metal ion concentration is the best predictor of a metal's bioavailability (Campbell 1995), the neutral  $\text{AgCl}^0$  complex can be highly bioavailable due to its nonpolarity and size (Engel et al. 1981). Dissolved Ag was absorbed by *D. polymorpha* with a slightly higher efficiency than in *M. edulis*—1.9% compared to 1.5% (Table 9). As with Ag, chlorocomplexes of Hg can penetrate membranes, and bioavailability of this metal, even in its inorganic form, may not be directly related to free metal ion activity (Mason et al. 1996). Of course, methylated forms of Hg can also be accumulated, but this was not evaluated in this study. Cd is also chlorocomplexed in seawater; however, the free

ion  $\text{Cd}^{2+}$  is generally the most bioavailable form (Campbell 1995), so we can account for a higher absorption efficiency in freshwater (*D. polymorpha*: 1.0%) than in seawater (*M. edulis*: 0.3%) (Table 9). The least absorbed elements, Cr(VI) and Se, both speciate as anions and are less reactive with solid phases, which typically carry negative surface charges at circumneutral pH values, possibly accounting for their lower absorption values in freshwater and marine mussels (Table 9). In fact, the only appreciable difference between *D. polymorpha* and *M. edulis* for uptake of Cr and Se was that both oxidation states of Cr were more highly absorbed by *D. polymorpha* than by *M. edulis*. In addition, Cr(III) was absorbed more efficiently than Cr(VI), the reverse of findings in seawater (Wang et al. 1997). Other work has also shown that cellular uptake from solution of Cr(III) is slower than that of Cr(VI) (Nieboer and Jusys 1988). No studies were performed to explain these differences, and it is not known whether this is due to differences in Cr speciation or to physiological differences between the mussels.

Previous studies that examined uptake of metals into zebra mussels from solution (Bias and Karbe 1985; Herwig et al. 1989) cannot be used to determine absorption efficiencies because their measurement requires short exposures and knowledge of the animal's ventilation (water pumping) rate. Ventilation rates of marine bivalves (Widdows and Hawkins 1989) and zebra mussels (Roditi et al. 1996) decline after a few hours in the absence of food; week- and month-long studies have starvation effects that cause the ventilation rates to decrease and be unpredictable. Further, absorption is difficult to assess if efflux during exposure is not negligible, and can easily be underestimated. Thus, short exposures are essential for the accurate determination of absorption efficiencies. Further, metal solute uptake shows a linear pattern over time in diverse invertebrates (Wang and Fisher 1998; Wang pers. comm.), and we would expect that longer exposure periods would produce comparable metal influx rates. Although absorption efficiencies are 1 to 2 orders of magnitude lower than AEs from food, uptake from the dissolved phase can contribute significantly to body burdens, particularly if a trace element is predominantly in the dissolved phase, which in turn is dependent on an element's particle reactivity and on environmental variables such as TSS loads and water chemistry. Clearly, metal influx rates from the dissolved phase are dependent on dissolved inorganic and organic ligands, which may vary spatially and temporally. The calculated uptake rate constants from our solute uptake experiments should therefore be extrapolated to other waters with caution. Among the factors that may influence bioavailability of a metal from the dissolved phase, metal complexation by dissolved organic matter has been recognized as critical (Campbell 1995).

*Trace element efflux rates*—Patterns of metal release from zebra mussels, in which depuration rates typically changed over time (that is, could be represented mathematically by belonging to more than one compartment), are characteristic of metal loss from diverse invertebrates, including other mussels (Fisher et al. 1996; Wang et al. 1996). The initial loss (first compartment) is dominated by egestion of radiolabeled food after food exposures and probably by desorp-

tion of loosely bound elements from tissue surfaces following dissolved exposures. The finding that efflux rates of metals from *D. polymorpha* were similar following different uptake pathways (food versus water) is consistent with the observations of Wang et al. (1996) for Am, Cd, Co, Se, and Zn in *M. edulis*. Reasons for the influence of exposure pathways on loss rates of Cr and Se are not apparent but presumably involve their physiological compartmentalization in the mussels. This can be illustrated in a similar case of Ag loss from *M. edulis* in which Ag obtained from food may bind with metal-rich granules as Ag<sub>2</sub>S in cells of the digestive tract lining and can subsequently be released into the gut lumen for egestion, whereas Ag obtained from the dissolved phase is stored in the kidney prior to excretion (George et al. 1986); thus different pathways could be expected to result in different depuration rates.

*Implications for monitoring*—Bioenergetic-based kinetic models have been used to evaluate the relative importance of dietary versus solute uptake pathways for contaminants in aquatic invertebrates, as well as to make site-specific predictions of contaminant concentrations in select invertebrate species (e.g., Wang et al. 1996). In this model, the metal concentration in an animal can be described as

$$C_{ss} = (k_u \times C_w) / (k_{ew} + g) + (AE_f \times IR \times C_f) / (k_{ef} + g), \quad (4)$$

where  $C_{ss}$  = trace element concentration in zebra mussel dry soft tissues at steady state ( $\mu\text{g g}^{-1}$ ),  $k_u$  = uptake rate constant from the dissolved phase ( $\text{L g}^{-1} \text{d}^{-1}$ ),  $C_w$  = dissolved trace element concentration ( $\mu\text{g L}^{-1}$ ),  $AE_f$  = trace element assimilation efficiency from ingested seston (percentage),  $IR$  = particle ingestion rate of mussels ( $\text{g g}^{-1} \text{d}^{-1}$ ),  $C_f$  = trace element concentration on ingested particles ( $\mu\text{g g}^{-1}$ ),  $k_{ew}$  = efflux rate constant following dissolved uptake ( $\text{d}^{-1}$ ),  $k_{ef}$  = efflux rate constant following food uptake ( $\text{d}^{-1}$ ), and  $g$  = growth rate constant ( $\text{d}^{-1}$ ) (assumed in calculations below to be negligibly small compared to  $k_e$  values). An example of how this model can use the kinetic parameters measured here for zebra mussels is given for Cd. For a site with a dissolved Cd concentration of  $12 \text{ ng L}^{-1}$  (based on our recent unpublished measurements in the Hudson), a TSS load of  $10 \text{ mg L}^{-1}$  and seston composition similar to that used in this study, and using a  $K_d$  of  $4 \times 10^4$  (Table 1), it can be calculated that 29% of the total Cd will be on particles and 71% in solution, corresponding to  $4.9 \text{ ng Cd L}^{-1}$  on particles (or  $0.49 \mu\text{g Cd g}^{-1}$  particles). Assuming an ingestion rate of  $0.4 \text{ g g}^{-1}$  tissue dry weight  $\text{d}^{-1}$ , and employing the  $AE$ ,  $k_u$ , and efflux rates measured here, we estimate a steady state Cd concentration of  $6.6 \mu\text{g g}^{-1}$  dry weight tissue in *D. polymorpha*, with food and water contributing 55 and 45%, respectively, to total body burden of Cd. This Cd body burden is within the range ( $0.4$ – $11.1 \mu\text{g g}^{-1}$ ) observed in NOAA's National Status and Trends zebra mussel program (O'Connor, pers. comm.). Kinetic modeling can further be used to quantify the influence of physiological parameters ( $AE$ ,  $k_u$ , efflux rates, or preingestive particle selection) and environmental parameters (metal concentration, TSS load,  $K_d$ , seston composition) on steady state body burdens, bioaccumulation factors, and the relative contributions of food and water to bioaccumulation.

As with marine mussels, concentration factors for a number of metals in *D. polymorpha* can vary spatially and temporally (Mersch et al. 1992). These variations can make ambiguous the interpretation of field data for metal concentrations in zebra mussels as indicators of ambient water concentrations. Clearly, it is important to evaluate (1) the relative importance of food and water as sources of metals for these animals and (2) the net metal bioaccumulation on metal-specific and site-specific bases (under a variety of environmental conditions) if these mussels are to be used as sentinel organisms for freshwater systems. By applying these data in a bioaccumulation model, it may be possible to explain the variability in bioconcentration factors for metals observed in zebra mussels. Our study shows that these animals are capable of accumulating metals from both food and water and that uptake is proportional to ambient metal concentrations. As such, these mussels behave similarly to marine mussels that have been successfully employed as bioindicators of coastal contamination.

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