

Uptake and loss kinetics of Cd, Cr and Zn in the bivalves *Potamocorbula amurensis* and *Macoma balthica*: effects of size and salinity

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ABSTRACT: Radiotracer studies were employed to quantitatively compare the biokinetics of uptake from the dissolved phase (influx rates) and loss (efflux) between 2 bivalves, *Potamocorbula amurensis* and *Macoma balthica*, and among the metals Cd, Cr and Zn. Effects of salinity on influx rate were evaluated in these 2 highly euryhaline species as were effects of animal size on uptake and loss. Metal speciation and biological attributes interacted to differentiate bioaccumulation processes among metals and between species. Influx rates of the 3 metals ($\mu\text{g g}^{-1}$ [dry wt] d^{-1}) increased linearly with dissolved metal concentrations. Influx rates of Zn in both clams were 3 to 4 \times those for Cd and 15 \times those for Cr. However, influx on the basis of free ion activities would be faster for Cd than for Zn. Relative influx rates among the metals were similar in the 2 bivalves. But, absolute influx rates of all 3 metals were 4 to 5 \times greater in *P. amurensis* than in *M. balthica*, probably because of differences in biological attributes (i.e. clearance rate or gill surface area). As salinity was reduced from 30 to 5 psu, the influx rate of Cd for *P. amurensis* increased 4-fold and that for *M. balthica* increased 6-fold, consistent with expected changes in speciation. However the influx rates of Cr in both clams also increased 2.4-fold over the same range, indicating a biological contribution to the salinity effect. Influx rates of Zn were not significantly affected by salinity. Weight specific metal influx rates ($\mu\text{g g}^{-1}$ [dry wt] d^{-1}) were negatively correlated with the tissue dry weight of the clams, but most rate constants determining physiological turnover of assimilated metals were not affected by clam size. The exception was the rate constant for Cd loss, which resulted in faster turnover in large *M. balthica* than in smaller clams. The rate constant of loss for *P. amurensis* increased in the order of Cd (0.011 d^{-1}) < Zn (0.027 d^{-1}) < Cr (0.048 d^{-1}). This was different from the hierarchy of rate constants for *M. balthica*: Zn (0.012 d^{-1}) < Cd (0.018 d^{-1}) < Cr (0.024 d^{-1}).

KEY WORDS: Uptake · Efflux · Cadmium · Chromium · Zinc · Bivalve · *Potamocorbula amurensis* · *Macoma balthica* · Size · Salinity

INTRODUCTION

Bivalves are an important component of the benthos in coastal ecosystems and are commonly used as bio-sentinel organisms to assess exposure of the benthos to metal contamination (Goldberg et al. 1983, O'Connor 1992, Brown & Luoma 1995). Bivalves concentrate many metals in their soft tissue (e.g. Jackim et al. 1977, Borchardt 1983, Fischer 1988) and the tissue metal levels represent a time-integrated response to bio-

available metal in food and water (bioaccumulation). In addition to the influence of metal concentration, bioaccumulation can be complicated by external environmental factors and internal biological processes (Luoma 1989, Rainbow et al. 1990, Wright 1995). Environmental factors include salinity, pH, redox potential, dissolved organic carbon, temperature, competing ions, and food availability (Sunda et al. 1978, Zamuda et al. 1985, Bjerregaard & Depledge 1994). Internal biological factors of importance include size, sex, reproductive stages, nutritional state, and seasonal growth cycles (Strong & Luoma 1981, Latouche & Mix 1982, Wang & Fisher 1997). The comparative influences of

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many of these factors are not fully understood. Recently, methodology has improved for quantifying processes influencing bioaccumulation. For example, Wang & Fisher (1997) showed for *Mytilus edulis* how the differences in rate constants of metal uptake from dissolved sources changed when body size and growth rate were different. Wang et al. (1996) showed how influx rates of metals changed with salinity and elimination of dissolved organic material from water. Quantitative understanding of how the internal and external factors affect metal bioaccumulation is critical to understanding the dose of metal that animals will experience under varying environmental conditions. This knowledge is a prerequisite for modeling bioaccumulation (Luoma & Fisher 1997) and for the use of biosentinel organisms in environmental monitoring strategies.

Bioaccumulation of metals is also species-specific and metal-specific. The species-specific aspects of bioaccumulation could result from difference in feeding mode (filter feeding vs deposit feeding), exposure routes (dissolved vs food or benthic vs pelagic food web) and duration of exposure, as well as internal processes such as storage, detoxification and loss (Jackim et al. 1977, Roesijadi & Robinson 1994, Wallace & Lopez 1996, Reinfelder et al. 1997). Experimental studies have shown, for example, differences in metal uptake between suspension feeding and deposit feeding bivalves exposed in the same metal-enriched system (Creelius et al. 1982, Bryan 1985) or between benthos that feed from the water column versus benthos that feed on the sediments (Hare et al. 1994, Warren et al. 1998). It is proposed that some organisms can internally maintain or regulate approximately constant tissue levels of selected elements (most commonly Zn), regardless of external metal concentrations (Rainbow et al. 1990 and references therein). The biological diversity of the process suggests that it is desirable to understand bioaccumulation in multiple species and to use multi-species approaches to evaluate environmental contamination. Comparison of bio-kinetics among species or among metals may be a profitable avenue for improving such understanding.

In this study, we compare influences of an important internal biotic factor (body size) and an important external environmental influence (salinity) on Cd, Cr and Zn bioaccumulation in 2 species of bivalves, *Potamocorbula amurensis* and *Macoma balthica*. An important goal is to evaluate differences in bioaccumulation between the species and among the metals. We restrict the present study to physiological influx of dissolved metals and loss kinetics. Influx from food is addressed in a separate paper (Lee & Luoma 1998).

Cadmium was chosen for study because it shows a strong contamination gradient in *Potamocorbula amu-*

rensis tissue over a wide salinity range in North San Francisco Bay (SFB) (Brown & Luoma 1995) and appears to be associated with adverse physiological effects on bivalves (Brown & Luoma unpubl.). Previous studies (Phillips 1976, 1977, Fischer 1986, Lin & Dunson 1993) have shown that Cd speciation is affected by salinity and Cd bioaccumulation is influenced by animal size and salinity. However, the quantitative influences of these factors have been described only for mussels (Wang et al. 1996). Zinc is also found in enriched concentrations in some bivalves in North SFB (e.g. *Macoma balthica*) but not in others (e.g. *P. amurensis*) (Brown & Luoma 1995). Zn is an essential element that may be physiologically regulated in some species (Rainbow et al. 1990); so, comparison of its physiological fluxes to that of Cd and Cr, and between the 2 bivalves, is of interest.

Chromium was chosen for study because its geochemistry contrasts with Cd and Zn. Large masses of dissolved Cr enter North SFB with river inflows (Abu-Saba & Flegal 1997) and industrial effluents (Luoma et al. 1990). Cr bioaccumulates in bivalves in contaminated environments, including SFB, although concentrations in animals in the bay are low compared to levels in sediments (Chassard-Bouchaud et al. 1989, Brown & Luoma 1995). The bioavailability of Cr is thought to be dependent upon its oxidation state. Among dissolved forms, Cr (III) occurs in considerably lower concentrations than Cr (VI) (Mayer 1988, Abu-Saba & Flegal 1997). It is thought that Cr must occur as Cr (VI) to cross biological membranes readily (Holdway 1988), although marine animals can accumulate Cr from a few types of Cr (III)-labeled food particles (Bremer et al. 1990, Decho & Luoma 1991). Despite its reputation as the most toxic form of the element, physiological fluxes of dissolved Cr (VI) have been quantified only by Wang et al. (1997) for mussels.

The 2 bivalves chosen for study are dominant benthic macrofauna in SFB and are used as biosentinel organisms for metal contamination studies (Luoma et al. 1985, Cain & Luoma 1990, Brown & Luoma 1995). *Potamocorbula amurensis*, a highly euryhaline filter feeder, is an invader species in SFB that has become the dominant benthic infauna in the bay (Carlton et al. 1990). *Macoma balthica*, a facultative deposit feeder, inhabits the intertidal zone of the bay and may be endemic to SFB or may have been introduced in the late 19th century (Meehan et al. 1989). The 2 clams show considerably different metal bioaccumulation patterns (Brown & Luoma 1995, unpubl., Lee & Luoma 1998). Brown & Luoma (1995) reported that Zn concentrations in *M. balthica* were responsive to environmental changes and were about 7 to 8 times those of *P. amurensis* at a mudflat where they co-occurred; Cd levels in *P. amurensis* were 3 times those in *M. balthica*

from the same site. In North SFB, Cd, Cr, and Ni levels in *P. amurensis* decreased with an increase in salinity. The biological and geochemical processes responsible for the differences in metal bioaccumulation patterns among metals and between 2 species are largely unknown (Decho & Luoma 1991, Lee & Luoma 1998).

MATERIALS AND METHODS

Experimental animals. Bivalves were collected from SFB 1 wk prior to experiments (October 1996). *Macoma balthica* were collected by hand from the Palo Alto mud flat, South SFB. *Potamocorbula amurensis* were collected from USGS Stn 4.1, North SFB (Brown & Luoma 1995) with a Van Veen grab. Clams were returned to the laboratory, gently scrubbed of adhering sediment and progressively acclimated to the experimental salinities (5, 10, 20, 25 and 30 psu) and temperature (10°C) over a 1 wk period. A culture of the phytoplankton *Rhodomonas salinas* was fed to clams during the acclimation period. The experimental seawater was prepared by diluting coastal seawater (34 psu) with ultra-clean distilled deionized water. Prior to use, the experimental seawater was filtered through a 0.22 µm filter cartridge.

Influx rate from the dissolved phase. To determine the effect of metal concentration on influx rates, both clam species were exposed to a range of Cd, Cr and Zn concentrations. Experimental medium was prepared by the addition of Cd and Zn standards, in dilute nitric acid, and Na₂CrO₄·4H₂O to 2 l of 0.22 µm filtered 20 psu seawater in 4 l polyethylene containers. The metal additions to seawater were 1, 5, 10, 50 or 100× (0.11 µg l⁻¹ [1 nM] Cd, 0.26 µg l⁻¹ [5 nM] Cr, and 0.65 µg l⁻¹ [10 nM] Zn). Radioisotopes were used as tracers of stable metals; each container received 180 kBq of carrier-free ¹⁰⁹Cd, 254 kBq of ⁵¹Cr (VI) and 159 kBq of carrier-free ⁶⁵Zn. Only Cr (VI) was used for uptake from dissolved phase because Cr (VI) dominates in >18 psu SFB seawater (Abu-Saba & Flegal 1995) and is preferentially accumulated by bivalves (Nieboer & Jusys 1988, Wang et al. 1997). The spiked Cd and Zn levels as radioisotopes were 2 orders of magnitude lower and the spiked Cr level as radioisotope and carrier was an order of magnitude lower than dissolved metal concentrations in SFB (Flegal et al. 1991). All isotopes were diluted in 0.1 N double distilled HCl. To neutralize acid added with isotopes, each container received ~300 µl of 0.1 N double distilled NaOH.

Clam species were exposed separately with 15 clams per metal treatment. Because of the hazards and constraints of working with large volumes of radioactive liquid, a single aquarium was used per treatment. Per species, clams were exposed for either 4 h (*Potamocor-*

bula amurensis) or 8 h (*Macoma balthica*) at 10°C. *M. balthica* filters water more slowly than *P. amurensis*. Radioactivity in experimental media was monitored periodically by sampling 5 ml of the exposure media. Following exposure, clams were removed from the media, rinsed with 0.22 µm filtered 20 psu seawater, grouped according to size (5 size classes) with 3 individuals per group, and the radioactivity was determined immediately. Clams were then dissected and the radioactivity in the soft tissue and shell was assayed separately. Soft tissue was then dried at 60°C and weighted.

Experiments to determine the influence of salinity on the influx of Cd, Cr (VI) and Zn into the clams followed the above protocol except that clams were exposed to 5 salinities (5, 10, 20, 25 and 30 psu) with the stable metal additions of 0.55 µg Cd l⁻¹, 1.3 µg Cr l⁻¹, and 3.25 µg Zn l⁻¹ above natural seawater concentrations.

Efflux rate of assimilated metals. The efflux rates of metals were determined from clams exposed to both metal-contaminated microbes (algae and bacteria) and metals in solution, followed by 3 wk depuration in unlabeled seawater. Algae and bacteria were radiolabeled with protocols similar to those described above. A phytoplankton, *Rhodomonas salinas*, was inoculated from a stock culture into 2 l of 0.22 µm filtered 20 psu seawater enriched with the f/2 nutrients (Guillard & Ryther 1962) minus Zn and EDTA. The medium was spiked with 363 kBq of ¹⁰⁹Cd, 407 kBq of ⁵¹Cr (III), 407 kBq of ⁵¹Cr (VI) and 317 kBq of ⁶⁵Zn. A common marine bacterium, *Pseudomonas atlantica*, was inoculated in 125 ml of 0.22 µm filtered 20 psu seawater containing 0.5% (w/v) dextrose and 0.2% bacto-peptone. The bacterium medium received 407 kBq of ⁵¹Cr (III) and 407 kBq of ⁵¹Cr (VI). Both Cr (III) and Cr (VI) were used to simulate the exposure of clams in natural estuarine conditions in which Cr in particulate phase is dominated by Cr (III) and in dissolved phase by Cr (VI) (Abu-Saba & Flegal 1995). Feeding cultures of Cr-labeled bacterial cells to clams was necessary to achieve enough radioactivity in the clam tissue, since Cr from bacterial cells is highly available by *Macoma balthica* and *Potamocorbula amurensis* (Decho & Luoma 1994, 1996), while Cr from most other food sources is much less bioavailable (Lee & Luoma 1998). Both cultures were incubated for 4 d on a light:dark cycle (14 h L:10 h D) in an 18°C incubation chamber. During the incubation culture flasks were shaken twice daily.

Following incubation, 1 l of radiolabeled algae and 60 ml of bacteria with the original labeled media were transferred directly into a 10 l aquarium containing 5 l of 0.22 µm filtered 20 psu seawater. Sixty clams of each species were exposed to this medium for 7 d at 10°C. On the 4th day of exposure the medium in the aquar-

ium was replaced with fresh 0.22 μm filtered 20 psu seawater and the remaining radiolabeled media (algae and bacteria). Following this 7 d exposure to radiolabeled food and water, clams were removed from the aquarium and rinsed of adhering particles with 0.22 μm filtered 20 psu seawater. The clams were grouped into 5 size classes with 4 individuals per group and an additional 8 randomly sized groups of 3 clams. Each group was immediately assayed for total radioactivity and 3 randomly sized groups of 3 clams were sacrificed to estimate the partitioning of radioactivity between the soft tissue and shell. The remaining groups were transferred to depuration chambers enclosed within a recirculating seawater system modified to remove radioisotopes in solution by activated charcoal filter. Clams were fed on unlabeled *Rhodomonas salinas* and were allowed to depurate the accumulated metal for 21 d.

During the depuration periods, each group was temporarily removed from the depuration chamber to measure total radioactivity. A randomly sized group of 3 individual clams was sacrificed on Days 1, 3, 7, 10 and 16 to determine the proportion of radioactivity partitioned to soft tissue. Following 21 d depuration, the remaining 5 size classes of 4 individual clams were measured for total radioactivity and, following dissection, re-assayed for radioactivity in tissue and shell. The temporal changes in the radioactivity of soft tissue for the clams were estimated by multiplying whole clam radioactivity by soft tissue/shell ratios obtained from clams that were dissected periodically. Clam tissues were then dried to constant weight at 60°C.

Radioactivity was determined with a gamma counter equipped with a well type NaI crystal detector. Photon emissions of ^{109}Cd were determined at 88 keV, ^{51}Cr at 320 keV and ^{65}Zn at 1115 keV. Counting times were ≤ 5 min and propagated counting errors were $< 5\%$. The raw data, cpm (counts per minute), were converted to dpm (disintegrations per minute) using appropriate standards and half-life corrections. Metal influx rates were expressed as $\mu\text{g metal g}^{-1}$ (dry wt) d^{-1} .

RESULTS

Influx rate from dissolved phase

The effects of concentration on influx rates of Cd, Cr and Zn ($\mu\text{g metal g}^{-1}$ [dry wt] d^{-1}) were expressed as a power function (Fig. 1):

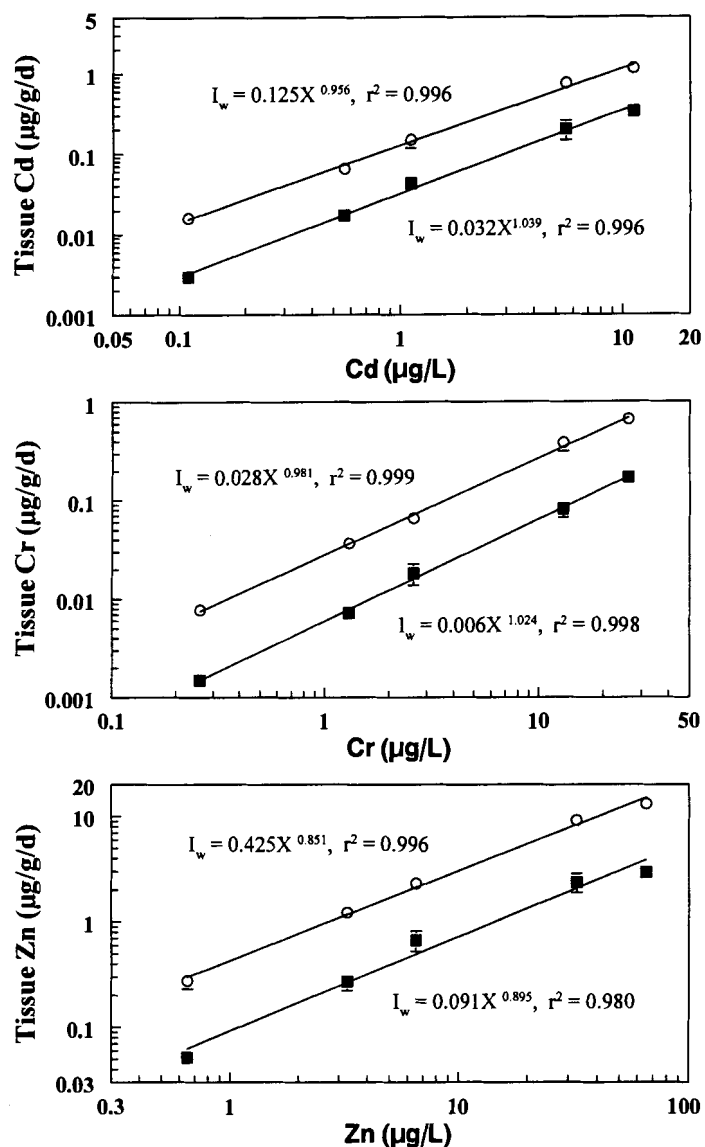


Fig. 1. *Potamocorbula amurensis*, *Macoma balthica*. Influx rates of Cd, Cr and Zn ($\mu\text{g g}^{-1}$ [dry wt] d^{-1}) into *P. amurensis* (O) and *M. balthica* (■) from the dissolved phase at different dissolved metal concentrations ($\mu\text{g l}^{-1}$). Values represent a mean (\pm SE) of 5 composite samples of 3 pooled clams. The dry weight of 3 pooled clams ranged from 0.038 to 0.130 g (with mean of 0.067 ± 0.028 g) for *P. amurensis* and from 0.089 to 0.451 g (with mean of 0.205 ± 0.090 g) for *M. balthica*. Log transformed metal influx rates (I_w) and metal concentrations (C_w) were linearly regressed

$$I_w = k_u C_w^b \quad (1)$$

where I_w is metal influx rate to clam tissue ($\mu\text{g metal g}^{-1}$ [dry wt] d^{-1}), k_u is the rate constant for uptake from the dissolved source, C_w is metal concentration in water and b (power coefficient) is the slope of the log-log relationship between I_w and C_w . The metal influx rates increased linearly ($p < 0.001$) with the exposure concentration (Fig. 1). The slopes (b) of all the relation-

ships were close to 1. The rate constant for uptake from the dissolved source (k_u) was metal- and species-specific and can be used as an indicator of bioavailability of dissolved metals (Wang et al. 1996). For all 3 metals, influx rates of *Potamocorbula amurensis* were 4 to 5 times greater than those of *Macoma balthica*. Both clams accumulated Zn 3 to 4 times faster than Cd. The influx of Cr to both clams was very slow compared to the other metals; it was 15 times slower than Zn influx rates and about 5 times slower than Cd influx rates.

The radioactivity partitioned between clam tissue and shell was relatively constant among the clams exposed to 5 levels of metals. The proportion of radioactivity partitioned in tissue to whole clam increased in the order of Cd ($16.0 \pm 1.8\%$) < Zn ($20.9 \pm 1.6\%$) < Cr ($46.0 \pm 3.5\%$) for *Potamocorbula amurensis* and Cd ($34.0 \pm 6.5\%$) < Zn ($34.6 \pm 3.3\%$) < Cr ($44.7 \pm 4.4\%$) for *Macoma balthica* (data not shown).

Salinity change affected most, but not all, influx rates. In both clams, influx rates of Cd and Cr increased significantly as salinity declined ($p < 0.001$; single classification ANOVA), especially at the lowest salinities (5 and 10 psu) (Fig. 2). As the salinity decreased from 30 to 5 psu, the Cd influx rate increased 4.0 times for *Potamocorbula amurensis* and 5.7 times for *Macoma balthica* and the Cr influx rates for both clams increased ~ 2.4 times. However, influx rates of Zn in *M. balthica* were not affected by salinity ($p > 0.1$). Influx rates of Zn for *P. amurensis* declined at 5 psu but otherwise were also unaffected by salinity. All influx rates were more variable at lower salinities, as evidenced by the higher standard errors around the mean. Similar to metal influx to soft tissue, the radioactivity partitioned to clam shells generally increased as the salinity decreased from 30 to 5 psu (Table 1). This increase was most pronounced for Cd and was about 5.5-fold for *M. balthica* and 2-fold for *P. amurensis*. The observed high standard deviations were due to the difference in size of the clams within each treatment.

To assess effects of clam size (dry wt) on metal influx rates, radionuclide uptake rates were pooled for all concentration treatments and plotted against dry wt (Fig. 3). It was possible to pool the data because influx rates were linearly related to metal concentrations in seawater, and all treatments initially received equal spikes of activity (i.e. all treatments were normalized to a similar initial activity). One exception was Zn, which had a slope (b) slightly less than 1 (Fig. 1). The relationship between weight-specific influx rate of 3 isotopes (I_i ; $\text{dpm g}^{-1} [\text{dry wt}] \text{d}^{-1}$) and dry wt of the clams (W) was also expressed as a power function (a power function best defines most size-related processes in bivalves; Nichols & Thompson 1982):

$$I_i = aW^c \quad (2)$$

where a is the intercept constant and c is the slope of the relationship between log transformed I_i and W . In *Macoma balthica*, the influx rates of all 3 isotopes decreased significantly ($p < 0.05$) as dry weight increased. The ^{109}Cd influx rate for *Potamocorbula amurensis* also had a significant inverse relationship ($p < 0.05$) with clam dry weight; no significant relationships were found between the influx rates of ^{51}Cr and ^{65}Zn and dry weight.

Clam size also affected radionuclide bioaccumulation after 7 d of exposure (the pre-efflux exposures) (Fig. 4). The slopes of the power function relating size

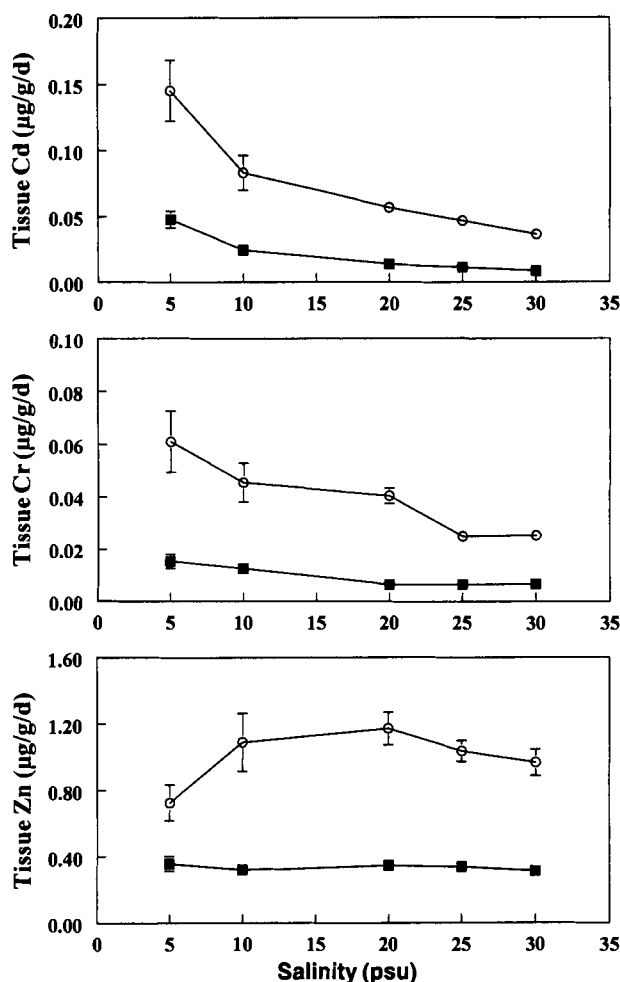


Fig. 2. *Potamocorbula amurensis*, *Macoma balthica*. Influx rates of Cd, Cr and Zn ($\mu\text{g g}^{-1} [\text{dry wt}] \text{d}^{-1}$) into *P. amurensis* (○) and *M. balthica* (■) from the dissolved phase at different salinities (psu). Values represent a mean (\pm SE) of 5 composite samples of 3 pooled clams. The dry weight of 3 pooled clams ranged from 0.046 to 0.167 g (with mean of 0.094 ± 0.040 g) for *P. amurensis* and from 0.111 to 0.416 g (with mean of 0.206 ± 0.086 g) for *M. balthica*

Table 1. Partitioning of radioactivity (dpm/1000) between tissue and shell of the clams used in the salinity experiment. Mean and standard deviation (SD) values were determined from 5 replicates of 3 pooled clams. These values were not corrected for clam size

Salinity (psu)		Cd (dpm/1000)		Cr (dpm/1000)		Zn (dpm/1000)	
		Tissue	Shell	Tissue	Shell	Tissue	Shell
<i>Potamocorbula amurensis</i>							
5	Mean	17.2	69.6	11.8	7.5	13.0	36.3
	SD	6.4	22.0	5.9	3.5	4.6	11.9
10	Mean	14.4	70.1	12.5	8.0	27.9	83.8
	SD	6.3	19.4	5.9	2.2	9.2	22.4
20	Mean	8.6	49.5	9.5	6.9	25.1	79.6
	SD	3.7	11.9	4.1	2.3	6.7	16.5
25	Mean	6.2	38.7	5.2	5.7	20.4	60.7
	SD	2.4	15.7	2.2	2.7	6.0	20.5
30	Mean	4.7	32.4	5.2	6.1	18.7	68.4
	SD	2.0	9.4	2.1	2.1	6.0	13.3
<i>Macoma balthica</i>							
5	Mean	25.2	55.4	12.4	6.3	28.9	59.5
	SD	5.1	13.1	2.1	1.9	5.8	11.7
10	Mean	15.9	30.8	13.2	5.2	32.5	54.8
	SD	6.0	9.1	5.6	2.2	13.4	13.8
20	Mean	8.5	17.0	6.4	4.4	33.2	49.2
	SD	2.1	4.6	1.7	1.7	7.6	8.3
25	Mean	7.1	11.5	6.7	4.7	33.1	46.9
	SD	2.2	3.9	2.3	1.1	11.2	8.3
30	Mean	5.3	9.8	6.8	4.7	31.3	45.4
	SD	1.1	3.7	2.1	1.5	10.4	7.8

and radionuclide accumulation (7 d dpm g⁻¹ [dry wt]) were negative for the 3 metals and for both clams, although *c* values for Cr and Zn in *Macoma balthica* were not different from zero ($p > 0.05$). Although Cr influx rates in *Potamocorbula amurensis* exceeded those in *M. balthica* (Fig. 1), Cr bioaccumulation after 7 d was less in *P. amurensis* than in *M. balthica* (Fig. 4). Bioaccumulation of Zn and Cd was greatest in *P. amurensis*, as were influx rates (Fig. 4).

Efflux rate of the assimilated metals

The metal accumulated during 7 d exposure to the radiolabeled food and water was gradually lost from the tissue during depuration (Fig. 5). The release was most rapid and variable during the first 2 d for *Potamocorbula amurensis* and first 4 d for *Macoma balthica*. This initial loss was largely due to defecation of unassimilated metal from the digestive tract. The gut residence time is ~24 h for *P. amurensis* and 72 to 96 h for *M. balthica* (Decho & Luoma 1991). The substantial loss of Cr during the first 4 d was the result of low assimilation of this metal compared to Cd and Zn (Lee & Luoma 1998).

The release of metals following defecation represents efflux from the tissue due to physiological turnover of assimilated metals (e.g. Fisher et al. 1996). The release of assimilated metals was slower and less variable among replicates than was defecation of unassimilated metal (Fig. 5). The physiological turnover of assimilated metals in clam tissue followed a first order exponential function (Fig. 6):

$$C_{(t)} = ae^{kt} \quad (3)$$

where $C_{(t)}$ is the fraction of metal retained in clam tissue at Day t , a is constant, k is rate constant of loss, and t is time (d). The rate constant of loss for *Pota-*

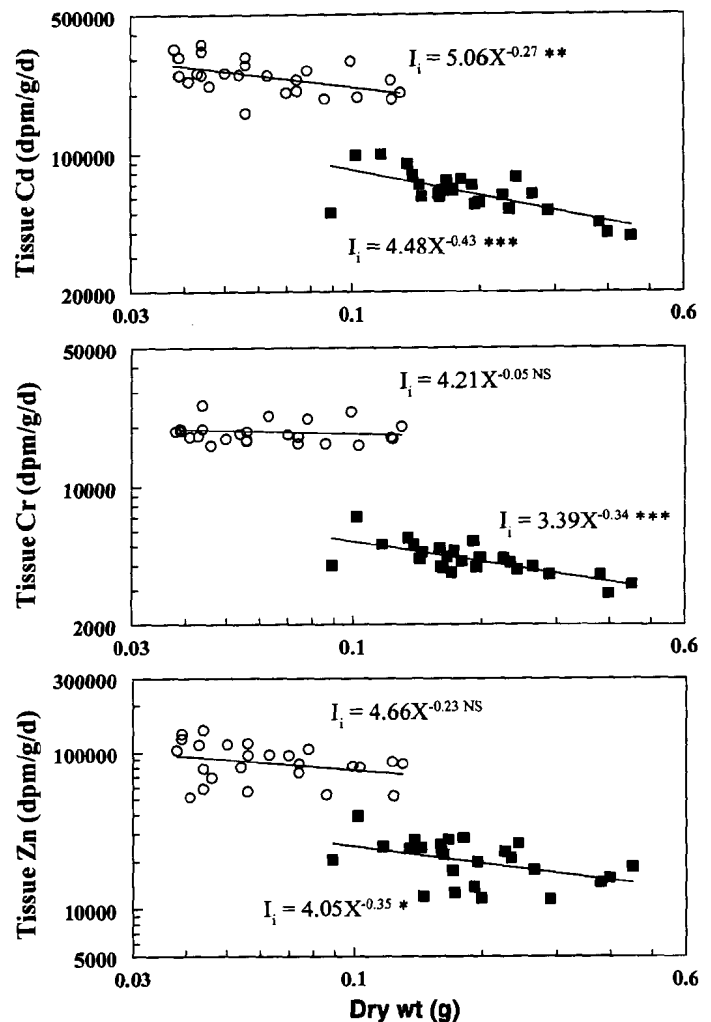


Fig. 3. *Potamocorbula amurensis*, *Macoma balthica*. Relationship of influx rates of ¹⁰⁹Cd, ⁵¹Cr and ⁶⁵Zn (dpm g⁻¹ [dry wt] d⁻¹) into *P. amurensis* (○) and *M. balthica* (■) from the dissolved phase with dry wt of 3 pooled clams (g). Log transformed isotope influx rates (I_1) and dry wt of clams (W) were linearly regressed. Regression lines were significantly different from zero at *0.01 < p < 0.05, **0.001 < p < 0.01, or *** p < 0.001; NS: not significant at p < 0.05

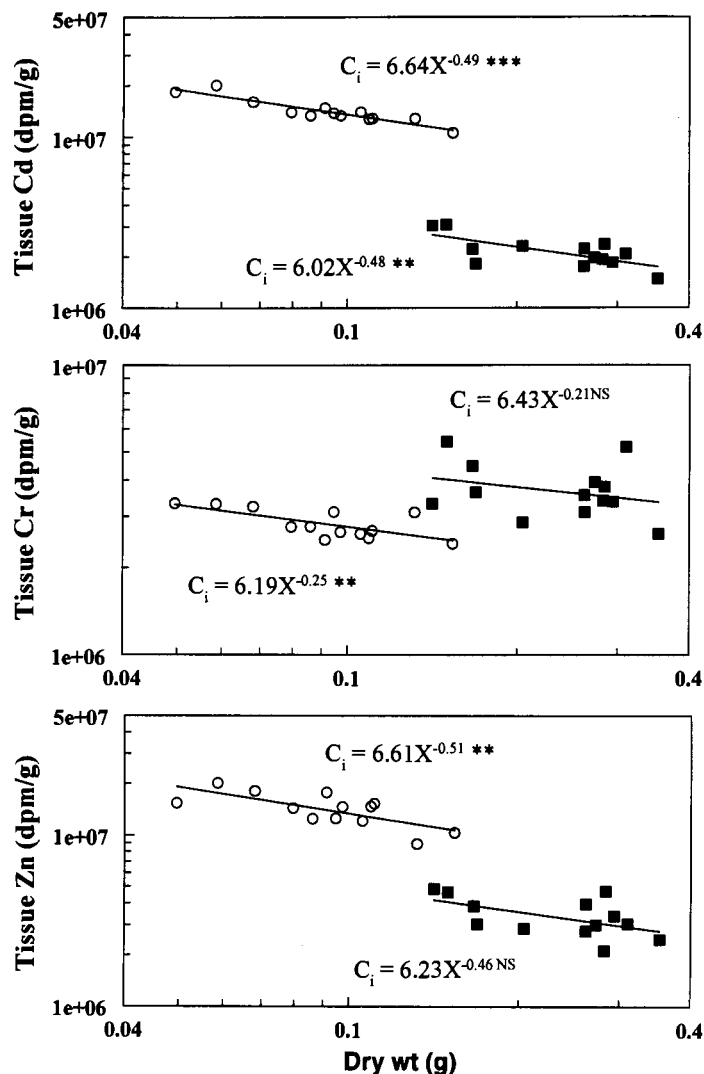


Fig. 4. *Potamocorbula amurensis*, *Macoma balthica*. Relationship of the tissue content of ^{109}Cd , ^{51}Cr and ^{65}Zn (dpm g^{-1} [dry wt]) and dry wt (g) of clams *P. amurensis* (○) and *M. balthica* (■) following exposure to radiolabeled algae and water for 7 d. Log transformed isotope content in clam tissue (C_i) and dry wt of clams (W) were linearly regressed. Regression lines were significantly different from zero at $**0.001 < p < 0.01$ or $***p < 0.001$; NS: not significant at $p < 0.05$

potamocorbula amurensis increased in the order of Cd ($0.011 \pm 0.002 \text{ d}^{-1}$) < Zn ($0.027 \pm 0.002 \text{ d}^{-1}$) < Cr ($0.048 \pm 0.002 \text{ d}^{-1}$). The rate constants of loss for *Macoma balthica* followed a different hierarchy: Zn ($0.012 \pm 0.001 \text{ d}^{-1}$) < Cd ($0.018 \pm 0.001 \text{ d}^{-1}$) < Cr ($0.024 \pm 0.001 \text{ d}^{-1}$). The differences in k values between 2 clams were statistically significant ($p < 0.05$ for Cd; $p < 0.001$ for Cr and Zn). Generally, tissue weight did not have a significant ($p < 0.05$) influence on the physiological turnover of metals in either clam, except for Cd in *M. balthica*. The rate constant of Cd loss in *M. balthica* was slightly greater ($p < 0.05$) in the 2 larger classes (0.025 d^{-1}) than in the 3 smaller groups (0.014 d^{-1}).

DISCUSSION

Influx rate among metals

The rate constant for dissolved metal uptake (k_u) is a metal- and species-specific measure of the bioavailability of dissolved metals. For a given total molar metal concentration, both clams accumulated Zn most efficiently, followed by Cd and then Cr (VI). Wang et al. (1996) reported that k_u values also increased in the order of Cr (VI) (0.1) < Cd (0.365) < Zn (1.044) in the blue mussel *Mytilus edulis*. For all 3 bivalves the relative k_u values among metals are similar (Zn [1]:Cd [1/3.5]:Cr [1/10 to 1/15]), although the absolute k_u values are different for each bivalve species (Fig. 1). This implies that relative transport, perhaps indicative of relative permeability through the gill membrane, is facilitated by a common mechanism(s) among all 3 bivalves.

It is relatively well established that free ion activity, rather than total metal concentration, determines the biological availability of dissolved metal, although there are some exceptions (Campbell 1995 and references therein). Because the proportion of free ion in seawater can be different among metals, relative bioavailability should be calculated based on the free ion rather than total metal concentrations. Although we did not directly determine speciation, some generalizations about free ion abundance are possible. For example, Cd speciation in seawater is dominated by weak Cl^- complexes. We can comfortably assume that free Cd^{2+} in our experimental seawater at 20 psu was a low percentage of the total: 5 to 7% based on chemical equilibrium models (Mantoura et al. 1978, Blust et al. 1992). In contrast, Mantoura et al. (1978) estimated that about 65% of dissolved Zn would be free ionic Zn^{2+} at 20 psu estuarine seawater. In South SFB, specifically, the fraction of free ionic Cd^{2+} was estimated to be in the relatively narrow range of 4 to 5% of total dissolved Cd, and was about 1/10 of the fractional abundance of Zn^{2+} (48%) (Wood et al. 1995). Such estimates could be modified if the partitioning of metals in our experimental media had been different due to organic complexation (Bruland 1989, 1992). Nonetheless, it is instructive to recalculate k_u if the fraction of free ion Zn exceeded that of free ion Cd by 10 \times . If the 20 psu experimental seawater contained 6% of total Cd as Cd^{2+} and 65% of Zn as Zn^{2+} , the k_u values based on free ion concentration would increase by 17 \times over the experimental Cd k_u but only by 1.5 \times more than the experimental Zn k_u . The k_u values for Cr (VI) would not change because it is likely

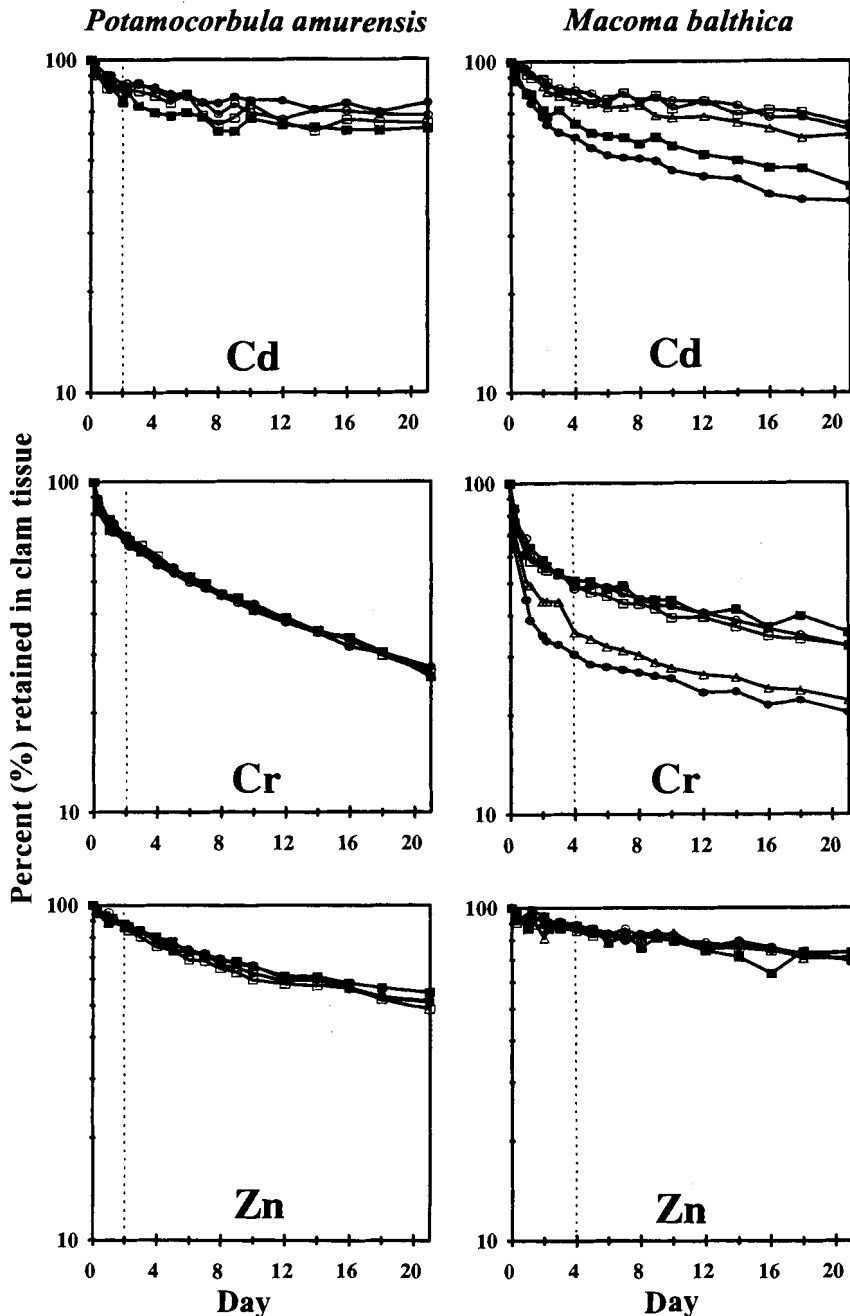


Fig. 5. *Potamocorbula amurensis*, *Macoma balthica*. Percentage of Cd, Cr and Zn retained in the soft tissue of *P. amurensis* (○) and *M. balthica* (■) during 21 d depuration following exposure to radiolabeled algae and water for 7 d. Symbols represent 5 size classes of 4 pooled clams (dry wt): 0.35 (■), 0.31 (●), 0.27 (Δ), 0.20 (□), and 0.14 g (○) for *P. amurensis* and 0.15 (■), 0.13 (●), 0.10 (Δ), 0.09 (□), and 0.08 g (○) for *M. balthica*. Each size class was composed of 4 individual clams. Physiological loss rates were calculated for the periods between Day 2 for *P. amurensis* or Day 4 for *M. balthica* (as indicated by the dotted lines) and Day

that all Cr would remain as Cr (VI) during the short influx experiments (Anderson et al. 1994). Based on free ion concentrations, the k_u for Cd would be about 3× and 70× the k_u values for Zn and Cr, respectively.

Thus the permeability of the gill membrane of bivalves to Cd^{2+} may be greater than that to Zn^{2+} . The oxianionic forms (CrO_4^{2-} , NaCrO_4^-) and anionic species seem to be much less available for bioconcentration from solution than the cations Cd^{2+} and Zn^{2+} .

Influx rates based on total dissolved metal concentration are conditional constants that will vary with metal speciation. Constants more directly indicative of physiological processes are obtained when influx rate is determined for a chemically defined circumstance or medium, i.e. based on free ionic metal concentration (Blust et al. 1992). This should be the next step in the development of the quantitative biokinetic bioaccumulation methodology.

Influx rate among species

Influx rates of the 3 metals (k_u) for *Potamocorbula amurensis* were 4 to 5 times greater than those for *Macoma balthica* (Fig. 1), but influx rates for both clams were slower than those reported for *Mytilus edulis* (Wang et al. 1996). Cd and Zn influx in *M. edulis* was about 11 times greater than in *M. balthica*; Cr (VI) influx in *M. edulis* was about 15 times greater than in *M. balthica*. These differences suggest that species-specific physiological attributes are one determinant of metal uptake. Attributes that could be important include clearance rate, gill surface area, or general permeability of the gill membrane to metal ions.

The linear relationship between influx rate and dissolved metal concentration is one indication that uptake of the 3 metals is a passively facilitated rather than actively regulated process (Roesijadi & Robinson 1994). Therefore, metal uptake rate could be influenced by the amount of water processed by the gills of the organisms (Janssen & Scholz 1979, Borchardt

1983, Rüsågård et al. 1987). The filtration rate of *Potamocorbula amurensis* (5.5 to 20.5 mm) in circulating flume conditions varied from 100 to 600 l g^{-1} (dry wt) d^{-1} over a free-stream velocity of 9 to 25 cm s^{-1} (Cole et

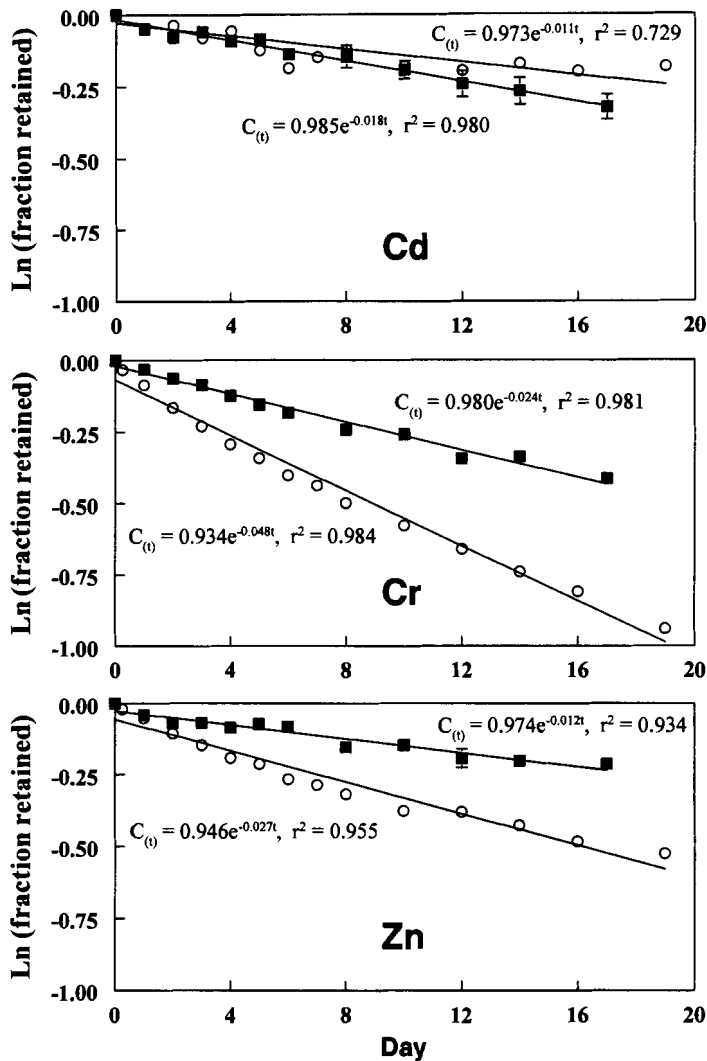


Fig. 6. *Potamocorbula amurensis*, *Macoma balthica*. Retention of assimilated Cd, Cr and Zn in the soft tissue of *P. amurensis* (○) and *M. balthica* (■) following depuration of unabsorbed digestive materials for 2 d (*P. amurensis*) and 4 d (*M. balthica*). Values represent mean (\pm SE) of 5 composite samples of 4 pooled clams. Linear regressions were made between the natural log transformed percent of retained metal ($C_{(t)}$) and depuration time (t)

al. 1992). Similarly, Werner & Hollibaugh (1993) reported that filtration rates of *P. amurensis* (10 to 20 mm) in a static system varied from 30 to 700 l g⁻¹ (dry wt) d⁻¹. Compared to *P. amurensis*, the filtration rate of *Macoma balthica* (20 to 21 mm) is orders of magnitude lower, ranging from 1 to 4 l g⁻¹ (dry wt) d⁻¹ (Harvey & Luoma 1984); that of a similar species, *Macoma nasuta* (25 to 32 mm), was 0.4 to 0.7 l g⁻¹ (dry wt) d⁻¹ (Winsor et al. 1990). The slower metal uptake rates observed from the facultative deposit feeder *M. balthica* are reasonable considering their slow clearance rate and less well developed gill structure (Gilbert 1977). Consistent with our observation, Jackim et al. (1977) reported that

after 3 wk exposure of 4 bivalves species to dissolved Cd, the filter feeders as a group (*Mya arenaria*, *Mytilus edulis*, and *Mulinia lateralis*) accumulated significantly more Cd than the deposit feeder *Nucula proxima*.

Filtration rates of mussels *Mytilus edulis* (42 mm) collected from the North Sea ranged from 52 to 196 l g⁻¹ (dry wt) d⁻¹ with a mean of 144 l g⁻¹ (dry wt) d⁻¹ (Widdows et al. 1995). Wang & Fisher (1997) reported that filtration of *M. edulis* (15 to 25 mm) varied from 82 to 570 l g⁻¹ (dry wt) d⁻¹. The differences in filtration rates between *M. edulis* and *Potamocorbula amurensis* are within the large variability of the measurements, so the role of clearance in differences among metal influx rates is difficult to determine. Determination of clearance rates simultaneously with metal influx rate might facilitate better understanding of this factor.

Effects of salinity

The inverse relationship between salinity and influx rates of Cd or Cr is consistent with previous field studies (Phillips 1977, Phelps et al. 1985), laboratory studies (Phillips 1976, Jackim et al. 1977, Fischer 1986, Wang et al. 1996) and toxicity bioassays (McLusky et al. 1986, Lin & Dunson 1993). Both geochemical and physiological mechanisms can be involved in the response of metal influx to salinity change (Wright 1995).

The most common geochemical explanation for increased metal uptake at low salinities is a change in speciation (Sunda et al. 1978, Blust et al. 1992, Wright 1995). The increasing influx of Cd at the lowest salinities, in both *Potamocorbula amurensis* and *Macoma balthica*, was consistent with such a response. The major dissolved Cd species in model estuarine waters are CdCl₂⁰, CdCl⁺, CdCl₃⁻, CdSO₄, and Cd⁺². Free Cd⁺² ion activity increases as salinity decreases, especially below salinities of 10 psu (Mantoura et al. 1978, Blust et al. 1992). Because *P. amurensis* and *M. balthica* are both tolerant of salinities as low as 5 psu (or lower in the case of *P. amurensis*) they may be exposed to more substantial changes in Cd speciation than are the less euryhaline mussels often employed in such studies (Bjerregaard & Depledge 1994, Wang et al. 1996). The significant increases in Cd adsorption on the clam shells as salinity decreases from 30 to 5 psu indirectly suggest increase in free ion activity at lower salinity waters.

At salinities below 10 psu, the reduction of Ca²⁺ concentration can also promote Cd influx by reducing com-

petition between Cd and Ca for the same transport sites (George & Coombs 1977). Reduced Ca^{2+} competition is apparently more important in some species than in others in increasing Cd uptake at low salinities (Wright 1977a, b, Bjerregaard & Depledge 1994). It cannot be eliminated as a factor in the increased influx of Cd in *Potamocorbula amurensis* and *Macoma balthica*. In contrast to Cd, changes in the speciation of Cr would not be expected at low salinities; Cr (VI) is stable in oxygenated seawater in the absence of particles (Anderson et al. 1994). Even if Cr (VI) was reduced to Cr (III) in lower salinity waters, Cr (III) influx rate to marine organisms is about 10-fold lower than Cr (VI) influx rate (Wang & Fisher 1997, Nieboer & Jusys 1988). Therefore, external Cr speciation changes were not a likely cause of the increased Cr influx at lower salinities. In estuarine waters, free ion Zn^{2+} dominates total dissolved Zn; more importantly, the proportion of ionic Zn^{2+} is relatively constant (~48%) in salinity ranges from 5 to 30 psu (Mantoura et al. 1978, Wood et al. 1995). Therefore increased Zn influx at lower salinities would not be expected from speciation changes, and was not observed in either *P. amurensis* or *M. balthica*.

Some of the above effects on metal influx could reflect physiological responses to changes in salinity. For example, Cr (VI) ion activity may increase at low salinity due to decreases in ionic strength, which could increase Cr (VI) permeation in the bivalves (Blust et al. 1992). Internal biological processes such as osmolarity changes or Ca^{2+} dependent changes in the permeability of the epithelial structures (George et al. 1978, Carpenne & George 1981) could also have contributed to twice faster Cr(VI) influx at 5 psu than at 30 psu. Changes in clearance rate also cannot be discounted as an internal biological influence. Consistent with our results, Wang et al. (1997) reported an inverse relationship between Cr (VI) influx rate in *Mytilus edulis* and salinity, but over a much smaller salinity range. Cd also could have been subjected to the same physiological/biological influences that apparently enhanced Cr uptake. Such responses would be additive with the response to increased free Cd ion activity. The failure of low salinities to increase Zn influx, in contrast with Cd and Cr, is contradictory to other studies that report increased Zn influx by crustaceans and molluscs with decreased salinity (e.g. Nugegoda & Rainbow 1989a, b, Wang et al. 1996). It is also not consistent with an overall increased gill permeability at lower salinities, which was one explanation for the Cr results. The discrepancy with Cr could be the result of different transport mechanisms for Cr and Zn on the gill epithelium. Cr (VI) is transported by anion transport mechanisms as phosphate or sulfate analogues, while Zn seems to be transported via facilitated diffusion process (Rainbow et al. 1990, Simkiss & Taylor 1995). Chan et al.

(1992) observed in the shore crab *Carcinus maenas* exposed to dissolved Zn in various salinities that haemolymph Zn levels were unaffected by or positively related to salinity. They explained that the reduction of Zn influx at lower salinity was due to reduction in water/electrotype permeability of gill epithelium. Such an effect would have to influence Zn and Cr differently to be operational in our experiments, however.

Effect of size

High variability among individuals characterizes the influence of animal size on bioaccumulation. However, a detectable negative influence of weight on instantaneous influx rates or short-term uptake is consistent with other laboratory studies (Fowler et al. 1978, Ringwood 1989, Wang & Fisher 1997). For example, Ringwood (1989) found in the exposure of the Hawaiian bivalve *Isognomon californicum* to dissolved Cd for 28 d that Cd accumulated in the larvae was about an order of magnitude greater than in adult bivalves. The effect was much smaller than that among the size classes of *Potamocorbula amurensis* and *Macoma balthica* employed in our study. Wang & Fisher (1997) found that influx rates of dissolved Cd, Co, Se and Zn in *Mytilus edulis* decreased with increasing body size (1.5 to 5 cm). The slopes of the power functions ranged from -0.3 to -0.6, values comparable to the *c* values for *P. amurensis* and *M. balthica* (-0.23 to -0.51). The dependence of metal influx rates on size is commonly explained by size-specific metabolic rates (Boyden 1974, Ringwood 1989, Newman & Heagler 1991). Wang & Fisher (1997) speculated that changes in gill surface area were also responsible for the size dependent metal influx rates displayed by differently sized mussels.

The difference in metal influx rates between the 2 clam species was not simply due to difference in animal size. The metal influx rates for *Potamocorbula amurensis* were greater than those for *Macoma balthica* when the relationships between metal influx rate and clam size were extended to the same size ranges (~0.1 g) (Fig. 3). A single relationship between physiological functions of many invertebrates and size has been suggested on the basis of the strong influence of size on weight-specific metabolism (Cammen 1980, Officer et al. 1982, Gerritsen et al. 1994). Fundamental differences in biology or physiology, beyond metabolic rate, must be invoked to explain the differences in metal influx between *P. amurensis* and *M. balthica*.

In contrast to laboratory studies which mostly show negative relationships between metal uptake and animal body size, studies relating tissue metal concentration and bivalve size in natural populations indicate

negative, neutral and positive relationships (Boyden 1974, 1977, Strong & Luoma 1981, Riget et al. 1996). One reason that negative relationships in natural populations are not as prevalent as implicated in the short-term laboratory studies is that the size dependent growth rates could offset the effects of size on metal influx rates (Strong & Luoma 1981, Wang & Fisher 1997). Generally, growth rate in smaller individuals is faster than in larger individuals (Blake & Jeffries 1971, Hamburger et al. 1983). Therefore, dilution of metal uptake by tissue growth could be a greater influence in smaller bivalves than in larger bivalves for a given species. Long-term changes in metal partitioning among intracellular components, with animal age, is another possible explanation that could be especially important in contaminated environments (Strong & Luoma 1981, Wallace unpubl.).

Efflux rates

Following depuration of unassimilated metals from the gut, the loss of the assimilated metals maintained a 1st order exponential pattern with a single compartment in both *Macoma balthica* and *Potamocorbula amurensis*. Other studies (Dahlggaard 1986, Wang et al. 1996) have described metal release patterns with more complex multi-compartment biokinetics, typically including rapidly exchanging pools and a slowly exchanging pool. The differences in the compartmental contributions to metal efflux will depend upon the duration of exposure (Cutshall 1974) as well as physiological considerations that could be metal- or species-specific, such as subcellular partitioning or pathways of physiological turnover among bivalve species. Overall the rate constant of metal loss from *P. amurensis* and *M. balthica* fell within the relatively narrow range of 0.01 to 0.05 d⁻¹. These values are within the ranges determined for other bivalves (*Mytilus edulis*, *Mercenaria mercenaria*, and *Crassostrea virginica*) (Wang et al. 1996, Reinfelder et al. 1997). However, the rate constants of loss from the bivalves in this and other studies are considerably lower than the values (0.07 to 0.3 d⁻¹) reported for crustacean zooplankton (Wang & Fisher 1998).

It is surprising that the rate constants of metal loss are not typically influenced by the size of bivalves (see also Wang & Fisher 1997 for Co, Se and Zn) if metabolic processes have a ubiquitous influence on metal biokinetic processes. Generally, rate constants of metal loss in bivalves are not influenced by the routes of metal exposure (Fisher et al. 1996), duration of exposure (Wang et al. 1996) and temperature (Hutchins et al. 1996, 1998) with some exceptions (Cutshall 1974, Wang et al. 1996). The lack of a temperature influence also raises questions about metabolic influences.

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