

# Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*

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**ABSTRACT:** Laboratory experiments employing radiotracer methodology were conducted to determine the assimilation efficiencies from ingested natural seston, the influx rates from the dissolved phase and the efflux rates of 6 trace elements (Ag, Am, Cd, Co, Se and Zn) in the mussel *Mytilus edulis*. A kinetic model was then employed to predict trace element concentration in mussel tissues in 2 locations for which mussel and environmental data are well described: South San Francisco Bay (California, USA) and Long Island Sound (New York, USA). Assimilation efficiencies from natural seston ranged from 5 to 18% for Ag, 0.6 to 1% for Am, 8 to 20% for Cd, 12 to 16% for Co, 28 to 34% for Se, and 32 to 41% for Zn. Differences in chlorophyll *a* concentration in ingested natural seston did not have significant impact on the assimilation of Am, Co, Se and Zn. The influx rate of elements from the dissolved phase increased with the dissolved concentration, conforming to Freundlich adsorption isotherms. The calculated dissolved uptake rate constant was greatest for Ag, followed by Zn > Am ≈ Cd > Co > Se. The estimated absorption efficiency from the dissolved phase was 1.53% for Ag, 0.34% for Am, 0.31% for Cd, 0.11% for Co, 0.03% for Se and 0.89% for Zn. Salinity had an inverse effect on the influx rate from the dissolved phase and dissolved organic carbon concentration had no significant effect on trace element uptake. The calculated efflux rate constants for all elements ranged from 1.0 to 3.0% d<sup>-1</sup>. The route of trace element uptake (food vs dissolved) and the duration of exposure to dissolved trace elements (12 h vs 6 d) did not significantly influence trace element efflux rates. A model which used the experimentally determined influx and efflux rates for each of the trace elements, following exposure from ingested food and from water, predicted concentrations of Ag, Cd, Se and Zn in mussels that were directly comparable to actual tissue concentrations independently measured in the 2 reference sites in national monitoring programs. Sensitivity analysis indicated that the total suspended solids load, which can affect mussel feeding activity, assimilation, and trace element concentration in the dissolved and particulate phases, can significantly influence metal bioaccumulation for particle-reactive elements such as Ag and Am. For all metals, concentrations in mussels are proportionately related to total metal load in the water column and their assimilation efficiency from ingested particles. Further, the model predicted that over 96% of Se in mussels is obtained from ingested food, under conditions typical of coastal waters. For Ag, Am, Cd, Co and Zn, the relative contribution from the dissolved phase decreases significantly with increasing trace element partition coefficients for suspended particles and the assimilation efficiency in mussels of ingested trace elements; values range between 33 and 67% for Ag, 5 and 17% for Am, 47 and 82% for Cd, 4 and 30% for Co, and 17 and 51% for Zn.

**KEY WORDS:** Bioaccumulation · Kinetic modeling · Metals · Mussels · Americium · Cadmium · Cobalt · Selenium · Silver · Zinc

## INTRODUCTION

Contamination of coastal waters by toxic chemicals is a major concern in many parts of the world. Measurements of ambient chemical concentrations in water or sediment have limited value for assessing the

potential impacts of contamination because these environmental pools may not represent the biologically available fraction. Consequently, marine mussels are employed throughout the world as biological monitors of coastal contamination. Field and laboratory studies have identified the attributes of suitable biomonitors (Phillips 1980) and the biological and abiotic factors that affect trace element bioaccumulation in such organisms (Phillips 1980, Bryan 1984). In the United

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States, the National Status and Trends (NS&T) Program monitors trace element concentrations in mussels and oysters from coastal regions (O'Connor 1992, 1996, O'Connor et al. 1994) in order to delineate contamination trends over large temporal and spatial scales.

The assumption in biomonitoring is that bivalves accumulate trace metals in direct proportion to ambient concentrations. There is evidence that this assumption is generally met for most metals (Phillips 1980). However, environmental conditions (such as suspended particle loads) and metal concentrations can vary in space and time on scales not easily measured in monitoring programs (e.g. spills, seasonal variability, locally contaminated sites such as near industrial outfalls) (Luoma et al. 1990). Models are necessary to supplement monitoring and predict bioaccumulation across this variety of conditions. In addition, it is difficult to unambiguously explain trends identified, for example, by NS&T, without a mechanistic understanding of the rates and routes of bioaccumulation. Models can help identify pathways and the most important processes responsible for contaminant bioaccumulation. Accurate bioaccumulation models are probably necessary to establish the key parameters for water quality criteria and provide an objective basis for risk assessment (DiToro et al. 1991, Luoma & Fisher 1996).

In the marine environment, trace elements are partitioned between aqueous and particulate phases, as controlled by processes such as adsorption/desorption and precipitation/dissolution. Marine bivalves are exposed to trace elements through the dissolved phase and ingestion of particulate matter. Element bioavailability is determined by geochemical behavior in both phases, as well as the biological factors that determine metal interactions with the animals. The relative importance of dissolved and particulate uptake vectors in trace element accumulation in aquatic animals has not been well characterized. Traditionally, this has been quantified by: (1) direct experimental separation of dissolved and particulate uptake; (2) indirect mass balance method to calculate the contribution of each uptake source; and (3) comparison of tissue distribution observed in the laboratory with observations in nature (Luoma 1989). Most of these experimental approaches have 2 major disadvantages: (1) experimental conditions often do not reflect those prevailing in natural waters, and (2) no single relationship is likely to be appropriate for all field conditions (therefore experiments are often not predictive).

Another approach to estimate bioavailability relies on bioconcentration factors, which relate metal concentration in the animals to a known ambient metal concentration. Equilibrium of metal partitioning between animal and aqueous environment is rarely met

in the laboratory; thus, it has been difficult to predict bioconcentration factors in the field from laboratory studies that assume equilibrium conditions. Recently, laboratory studies have focused on the kinetics of toxicant accumulation and depuration in aquatic animals, and several kinetic bioaccumulation models have been developed (Breck & Bartell 1988, Landrum et al. 1992, Newman 1995). Kinetic models have large data requirements, but the approach is not constrained by assuming that toxicant accumulation is by constant exposure or governed by thermodynamic equilibria (Landrum et al. 1992, Luoma & Fisher 1996). Kinetic models have been successfully applied to study contaminant bioaccumulation in marine bivalves when measurements are limited to a few important physiological parameters, including influx rate from dissolved phase, assimilation efficiency (AE) from ingested food particles, and efflux rates. For example, Boese et al. (1990) demonstrated that tissue concentrations of hexachlorobenzene in the clam *Macoma nastata* predicted from this model were very close to the observed tissue concentrations during a short-term experiment. Luoma et al. (1992) showed that the tissue concentration of Se predicted from this model was comparable to the actual Se concentration found in *Macoma balthica* in San Francisco Bay (California, USA).

In this paper, physiological parameters necessary to construct a kinetic trace element bioaccumulation model for mussels are identified and quantified. Parameters include trace element AEs from ingested natural seston, dissolved influx rates and efflux rates. Some of the factors affecting these processes are also considered. The model is then employed to predict trace element concentrations in mussel tissues under environmental conditions characteristic of San Francisco Bay and Long Island Sound (New York, USA), and the predicted concentrations are compared to concentrations observed in mussels in those locations. The goals of the modeling are to quantitatively characterize the processes responsible for metal accumulation in mussels in these and other estuarine systems, and to separate the contributions of different uptake vectors (dissolved vs particulate) to the overall metal uptake in mussels. Among the 6 trace elements studied in this paper (Ag, Am, Cd, Co, Se and Zn), elevated concentrations of Ag, Cd, Se and Zn are common in many coastal waters, whereas radioisotopes of Am and Co are important components of some nuclear wastes discharged into some coastal waters (Park et al. 1983, Mount et al. 1994). Of these elements, Co, Se, and Zn are biologically essential. Ag is a Class B (sulfur-seeking) metal, Am is a Class A (oxygen-seeking) metal, and the others are borderline metals (Nieboer & Richardson 1980).

## MATERIALS AND METHODS

Mussels *Mytilus edulis* of 3.0 to 3.5 cm shell length were collected from Oldfield, Long Island Sound, a location with no known elevated metal concentration in seawater (O'Connor 1992). Mussels were acclimated in 10 l of aerated seawater (28‰, 15°C) for 1 wk prior to experiments. During these periods they were continuously fed the diatom *Thalassiosira pseudonana* at a daily ration of about 2% of their tissue dry weight.

**Trace element assimilation from natural seston.** Water samples were collected from Stony Brook Harbor (SB) during the spring phytoplankton bloom (March 4 and 11, 1995). The chlorophyll *a* (chl *a*) concentrations in these waters were determined (Parsons et al. 1984) and phytoplankton abundance and composition were quantified microscopically. The seawater was first passed through a 160 µm nylon mesh to remove large particles and grazers, and then filtered through a 3 µm polycarbonate membrane. The particles (in the size range 3 to 160 µm) retained on the membrane were resuspended and concentrated into 2 flasks containing 120 ml of 0.2 µm filtered seawater (28‰).

Two groups of resuspended seston were then radiolabeled for 36 h with  $^{241}\text{Am}+^{75}\text{Se}+^{65}\text{Zn}$  and  $^{110\text{m}}\text{Ag}+^{109}\text{Cd}+^{57}\text{Co}$ , respectively, during which the sealed flasks were placed in the dark on a spinning wheel (2 rpm) to diminish aggregation of seston and prevent settling (Lee & Fisher 1994). Radioisotope additions were 37 kBq (320 nM) for  $^{110\text{m}}\text{Ag}$  (in 0.1 N  $\text{HNO}_3$ ), 37 kBq (120 nM) for  $^{241}\text{Am}$  (in 3 N  $\text{HNO}_3$ ), 74 kBq (43 nM) for  $^{109}\text{Cd}$  (in 0.1 N  $\text{HCl}$ ), 18 to 37 kBq (8.6 to 17.2 pM) for  $^{57}\text{Co}$  (in 0.1 N  $\text{HCl}$ ), 74 kBq (4.1 nM) for  $^{75}\text{Se}$  ( $\text{Na}_2\text{SeO}_3$ , in distilled water), and 74 kBq (100 nM) for  $^{65}\text{Zn}$  (in 0.1 N  $\text{HCl}$ ). Before the additions of radioisotopes, microliter amounts of 0.5 N Suprapur  $\text{NaOH}$  were added to maintain the pH of the seawater at 8.0.

At the end of radiolabeling, the seston was collected by filtration onto 3 µm polycarbonate membranes and resuspended into unlabeled 0.2 µm filtered seawater. Five individual mussels were placed in 800 ml of 0.2 µm filtered seawater held in a polypropylene beaker. Every 5 min radioactive seston was added at a concentration of 1.0 mg  $\text{l}^{-1}$ . Because mussels essentially ingested all particles during the 30 min radioactive feeding period, an average ingestion rate of 1.9 mg  $\text{h}^{-1}$  was achieved for each mussel. There was little variation in ingestion activity among different individual mussels. After the radioactive feeding, mussels were rinsed and radioassayed non-destructively (described below). Counting times were 1.5 min for each mussel. Mussels were subsequently placed individually into 240 ml polypropylene beakers, each containing 150 ml of seawater, and held within an enclosed recirculating

seawater system (described in Wang et al. 1995). The water flow rate through each mussel chamber was about 2  $\text{l h}^{-1}$ .

Previous experimentation indicated that mussels complete their digestion and assimilation of trace elements within 72 h (Wang et al. 1995). Consequently, mussels were depurated of the ingested radiolabeled materials for 4 d, during which they were continuously fed unlabeled diatom *Thalassiosira pseudonana* cells at an approximate daily ingestion rate of 3% of their tissue dry weight. Radioactivities of mussels were counted at time intervals of 4 to 12 h. Feces were collected throughout the depuration period and radioassayed. Because egestion of radiotracers occurred primarily within the first 12 h of depuration, feces were collected at time intervals of 1 to 3 h and later extended to every 4 to 12 h.

Aliquots of radiolabeled seston were analyzed to determine the cytoplasmic distribution of radiotracers, as described in Fisher et al. (1983b). Seston was collected on a 1 µm polycarbonate membrane and washed for 2 min with 10 ml of 1 mM EDTA to remove surface bound metals. The seston was then resuspended into distilled water (pH = 7.0) and frozen. Microscopic examination confirmed that all algal cells were broken apart by this treatment. Cell debris was centrifuged sequentially at different speeds to fractionate the cytological components of radiotracers (8000 × *g* for 15 min, 20000 × *g* for 15 min and finally 100 000 × *g* for 20 min). The final supernatant was considered to contain the cytoplasmic fraction.

**Trace element influx rate from the dissolved phase.** Dissolved influx rate describes the rate at which a trace element in the dissolved phase is accumulated by the mussel. Short exposures are employed to measure gross influx rates, the goal being to obtain a tissue concentration that is minimally influenced by efflux. Further, a mussel's ventilating activity will decline in the absence of food particles, and the influx rate may therefore be underestimated. Short exposures can overcome this difficulty. In our experiments, mussels were therefore exposed to dissolved trace elements for 2 h, during which the ventilation rate does not decline appreciably due to the absence of food particles (e.g. Widdows & Hawkins 1989).

Prior to these experiments, mussels were continuously fed with the diatom *Thalassiosira pseudonana* for 7 d. They were then placed individually into 500 ml of 0.2 µm filtered seawater (collected 8 km off Southampton, Long Island) with different dissolved concentrations of trace elements (stable plus radioisotope, with the exception of  $^{241}\text{Am}$ ). The experimental temperature was 15°C and the salinity of the experimental water was adjusted to 28‰. There were 8 replicate individuals for each treatment. Stable trace element



was added so that the lowest concentration was 3 to 5 times its typical concentration in uncontaminated coastal waters (with the exception of Ag and  $^{241}\text{Am}$ ). Thus, concentrations ranged from 0.2 to 100  $\mu\text{g l}^{-1}$  for Ag (in  $\text{AgNO}_3$ ); 0.1 to 10  $\mu\text{g l}^{-1}$  for Cd (in  $\text{CdCl}_2$ ), Co (in  $\text{CoCl}_2$ ) and Se (in  $\text{Na}_2\text{SeO}_3$ ), 0.5 to 300  $\mu\text{g l}^{-1}$  for Zn (in  $\text{ZnCl}_2$ ); and 9.4 to 247  $\text{ng l}^{-1}$  for  $^{241}\text{Am}$ . Radioisotopes were allowed to equilibrate with stable elements overnight prior to contact with the mussels.

Any feces egested by the mussels during the 2 h uptake period was immediately pipetted out of the water to minimize fecal scavenging of trace elements. The water was gently stirred every 10 min. After 2 h of uptake, mussels were rinsed and dissected. Radioactivities of tissues were measured with an LKB Compugamma counter (described below). The influx rate was calculated as the amount of trace element accumulated by the mussel and was normalized per gram dry wt per day ( $\mu\text{g g}^{-1} \text{d}^{-1}$ ).

The effects of ambient salinity on trace element uptake by mussels from the dissolved phase were experimentally determined. Mussels (from a salinity of 27‰) were acclimated to different salinities (20, 27, 34‰) for 10 d to minimize differences in clearance rates under different salinities (Widdows 1985). To acclimate to 15‰, the lowest salinity that did not disturb mussel behavior, mussels were first acclimated to 20‰ for 3 d and then adjusted down to 15‰ for 7 d. During these acclimation periods, mussels were fed with the diatom *Thalassiosira pseudonana*. Experiments followed the dissolved influx protocol described above. To minimize variation between water batches due to varying dissolved organic matter concentrations, one batch of Southampton surface water was used throughout the experiments, diluted with deionized water as appropriate (with pH checked). Stable trace element additions were 0.5  $\mu\text{g l}^{-1}$  each for Cd (in  $\text{CdCl}_2$ ), Co (in  $\text{CoCl}_2$ ), and Se (in  $\text{Na}_2\text{SeO}_3$ ), and 2  $\mu\text{g l}^{-1}$  for Zn (in  $\text{ZnCl}_2$ ). For Ag and Am, only  $^{110\text{m}}\text{Ag}$  (in 0.1N  $\text{HNO}_3$ ) and  $^{241}\text{Am}$  (in 3N  $\text{HNO}_3$ ) were added to result in dissolved concentrations of 0.256  $\mu\text{g l}^{-1}$  and 58  $\text{ng l}^{-1}$ , respectively.

Additional experiments were conducted to assess the influence of dissolved organic matter (DOM) on the influx rate of dissolved trace elements into mussels. These experiments used Southampton seawater (SA) and water collected from Stony Brook Harbor (SB). The salinity of the Southampton water was adjusted to 27‰ (the salinity of SB water). After adding  $\text{H}_2\text{O}_2$  (0.8% by volume), batches of seawater were irradiated with high intensity ultraviolet radiation (1200 W, Ace Hanovia Lamp, Ace Glass Co, Vineland, NJ, USA) to photooxidize the DOM (Armstrong et al. 1966). DOM concentrations in UV-oxidized seawater and unirradiated seawater were measured with a Shimadzu TOC

5000 analyzer. The pH of seawater did not change after UV oxidation. Prior to experiments, all glassware for these experiments was baked at 450°C for 5 h.

Both UV-oxidized and unirradiated seawater were filtered through 0.2  $\mu\text{m}$  polycarbonate membranes. Stable trace elements and radiotracers were then added to give a stable concentration of 1.0  $\mu\text{g l}^{-1}$  for Cd, Co and Se, and 2  $\mu\text{g l}^{-1}$  for Zn. For Ag and Am, only  $^{110\text{m}}\text{Ag}$  and  $^{241}\text{Am}$  were added, resulting in dissolved concentrations of 0.256 and 0.058  $\mu\text{g l}^{-1}$ , respectively. The influx rate of mussels was then determined as described above.

**Trace element efflux rates.** The diatom *Thalassiosira pseudonana* was radiolabeled with  $^{110\text{m}}\text{Ag}+^{109}\text{Cd}+^{57}\text{Co}$ , or  $^{241}\text{Am}+^{75}\text{Se}+^{65}\text{Zn}$ , respectively, following protocols described in Wang & Fisher (1996a). Radioisotope additions were 41  $\text{kBq l}^{-1}$  for all 6 radioisotopes, corresponding to 42.5 nM for  $^{110\text{m}}\text{Ag}$  (in 0.1 N  $\text{HNO}_3$ ), 15.9 nM for  $^{241}\text{Am}$  (in 3 N  $\text{HNO}_3$ ), 2.9 nM for  $^{109}\text{Cd}$  (in 0.1 N  $\text{HCl}$ ), 2.4 pM for  $^{57}\text{Co}$  (in 0.1 N  $\text{HCl}$ ), 0.3 nM for  $^{75}\text{Se}$  (as  $\text{Na}_2\text{SeO}_3$  in distilled water), and 6.7 nM for  $^{65}\text{Zn}$  (in 0.1 N  $\text{HCl}$ ).

Following 4 d growth, diatom cells had undergone >5.5 divisions and reached a concentration of  $1.8 \times 10^6$  cells  $\text{ml}^{-1}$  (for  $^{110\text{m}}\text{Ag}+^{109}\text{Cd}+^{57}\text{Co}$  treatment) and  $2.1 \times 10^6$  cells  $\text{ml}^{-1}$  (for  $^{241}\text{Am}+^{75}\text{Se}+^{65}\text{Zn}$  treatment), respectively. Cells were presumed to be uniformly radiolabeled and were collected onto polycarbonate membranes, rinsed with filtered seawater, and resuspended into 200 ml filtered unlabeled seawater. They were stored at 4°C for subsequent radioactive feeding of mussels.

Five individual mussels were placed in 2 l of 0.2  $\mu\text{m}$  filtered seawater. Each day, mussels were fed twice with radioactive diatoms. During each radioactive feeding period (lasting 3 to 4.5 h), algae were added into a mussel's feeding chamber every 1.5 h to produce a suspension of  $4.5 \times 10^4$  cells  $\text{ml}^{-1}$ . The water was aerated gently and replaced each day. After the radioactive feeding, mussels were transferred to 2 l of unlabeled seawater. Mussels were repeatedly fed under these conditions for 7 d. The contribution of accumulated radioisotope in mussels from the dissolved phase during the feeding period (following desorption from radioactive diatoms) was <4% of the total body burden for  $^{110\text{m}}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{75}\text{Se}$  and  $^{65}\text{Zn}$ , about 11% of the body burden for  $^{109}\text{Cd}$ , and 22% for  $^{57}\text{Co}$ .

After 7 d of radioactive feeding, mussels were rinsed with filtered seawater and their radioactivity was counted. They were then transferred individually into 240 ml polypropylene chambers within an enclosed recirculating aerated seawater system, as described above, and were allowed to depurate their incorporated radioisotopes for 20 d. The aquarium water was replaced every week to minimize accumulation of dis-

solved isotopes and mussel metabolites in the water. Radioactivities in the dissolved phase in this recirculating water were typically not detectable above background levels throughout the 20 d depuration period. The radioactivity of each mussel was periodically counted; note that gamma analysis is non-destructive, so the same individual mussels could be counted throughout the course of an experiment. Fecal pellets were collected every 2 to 8 h to minimize desorption of radiotracers from egested feces into the water. At the end of depuration, mussels were dissected and radioactivities associated with shell, gill, food, adductor muscle, digestive gland and remaining soft tissues were determined.

Additional experiments were conducted to assess the efflux rates following uptake of trace elements from the dissolved phase. Two groups of mussels (10 individuals each) were each placed in 2 l of 0.2 µm filtered seawater and exposed to dissolved  $^{110m}\text{Ag}$ ,  $^{109}\text{Cd}$ ,  $^{57}\text{Co}$  and  $^{241}\text{Am}$ ,  $^{75}\text{Se}$ ,  $^{65}\text{Zn}$ . Any feces egested by the mussels was collected with a trap held on the bottom of the beaker. Radioisotope additions were 3.7 kBq l<sup>-1</sup> for all 6 radiotracers, corresponding to 3.19 nM for  $^{110m}\text{Ag}$ , 1.19 nM for  $^{241}\text{Am}$ , 0.22 nM for  $^{109}\text{Cd}$ , 0.18 pM for  $^{57}\text{Co}$ , 0.02 nM for  $^{75}\text{Se}$ , and 0.51 nM for  $^{65}\text{Zn}$ . Each day, mussels were exposed to radioactive seawater for 18 h, and then transferred to unlabeled seawater for 6 h, during which they were fed on unlabeled diatoms. The seawater for radioactive exposure was replaced every day. Throughout the 6 d uptake period, only a small fraction of radioisotope (<2%) was associated with the particulate phase (>1 µm). After 6 d exposure, mussels were rinsed with filtered seawater and their radioactivity determined. They were then transferred into the enclosed recirculating system and depurated for 18 d, as described above.

A parallel experiment to this 'long-term' exposure experiment was conducted to assess efflux rates of trace elements obtained from the dissolved phase following short-term exposure, following the same protocol except that exposure was for only 12 h. Periodically, 2 replicate mussels were sacrificed and the radioactivities in the shell, digestive gland, gill, and remaining soft tissues were measured.

**Analytical measurements.** The radioactivity of whole mussels was assayed non-destructively with a large-well NaI (TI) gamma detector. Radioactivity of dissected mussel tissues, feces and labeled food particles were measured with a Pharmacia-Wallac LKB gamma counter equipped with a well-type NaI (TI) crystal. Spillover of radioisotopes was corrected and the 2 gamma counters were intercalibrated. All counts were related to standards for each isotope and corrected for radioactive decay. The gamma emissions of

$^{110m}\text{Ag}$  were determined at 658 keV, of  $^{241}\text{Am}$  at 60 keV, of  $^{109}\text{Cd}$  at 88 keV, of  $^{57}\text{Co}$  at 122 keV, of  $^{75}\text{Se}$  at 264 keV, and of  $^{65}\text{Zn}$  at 1115 keV. Counting times in all samples were adjusted so that the propagated counting errors were typically <5%.

**The bioaccumulation model.** Trace elements are available to mussels through both dissolved and particulate phases. Bioaccumulation via more than 1 pathway can be described with a kinetic model (Thomann 1981, Landrum et al. 1992, Thomann et al. 1995), assuming that uptake from each source is proportional to its influx. This can be described as:

$$dC/dt = \alpha_w \times FR \times C_w + \sum_{i=1}^n (AE \times IR \times C_i) - (k_e + g) \times C \quad (1)$$

where  $C$  is the trace element concentration in mussel soft tissues (µg g<sup>-1</sup>),  $t$  is the time of exposure (d), and  $\alpha_w$  is the trace element absorption efficiency from the dissolved phase,  $FR$  is the mussel filtration rate (l g<sup>-1</sup> d<sup>-1</sup>),  $C_w$  is the dissolved trace element concentration (µg l<sup>-1</sup>),  $AE$  is the trace element assimilation efficiency (%) from ingested particles,  $IR$  is the ingestion rate of mussels (mg g<sup>-1</sup> d<sup>-1</sup>),  $C_i$  is the trace element concentration in ingested particles (µg mg<sup>-1</sup>),  $j$  is the food compartment,  $k_e$  is the efflux rate constant (d<sup>-1</sup>), and  $g$  is the growth rate constant (d<sup>-1</sup>), assuming a first order growth. Throughout this study, sample weights are expressed on a dry weight basis.

Under steady-state conditions, Eq. (1) becomes:

$$C_{ss} = \frac{\alpha_w \times FR \times C_w + \sum_{i=1}^n (AE \times IR \times C_i)}{k_e + g} \quad (2)$$

where  $C_{ss}$  is the trace element concentration in mussel soft tissues (µg g<sup>-1</sup>) at steady state.

Because some of the parameters in Eq. (2) are not available, several modifications can be made:

$$I_u = \alpha_w \times FR \times C_w = k_u \times C_w \quad (3)$$

where  $I_u$  is the influx rate from the dissolved phase (µg g<sup>-1</sup> d<sup>-1</sup>), which can be measured experimentally, and  $k_u$  is the dissolved uptake rate constant (l g<sup>-1</sup> d<sup>-1</sup>), which equals  $\alpha_w \times FR$ . Our experiments (see 'Results') show that  $I_u$  is directly proportional to  $C_w$ , thus  $k_u$  can be computed from these measurements. When  $C_i$  is not known, it can be estimated from  $C_w$  by applying a partition coefficient and the dissolved concentration of a trace element:

$$C_i = C_w \times Kd \quad (4)$$

where  $Kd$  is the partition coefficient of a trace element on the ingested particles (l kg<sup>-1</sup>).

For most trace elements, the efflux rates following uptake from the dissolved phase and particulate inges-

tion are roughly comparable in mussels (see 'Results'). However, if efflux rates are dependent on the routes of uptake, Eq. (2) can be rewritten as:

$$C_{ss} = \frac{k_u \times C_w}{k_{ew} + g} + \frac{\sum_{j=1}^n (AE \times IR \times C_w \times Kd)}{k_{ef} + g} \quad (5)$$

where  $k_{ew}$  is the efflux rate constant after uptake from the dissolved phase and  $k_{ef}$  is the efflux rate constant after uptake from ingested particles.

Metal concentrations obtained from the dissolved phase and from food uptake can be described by:

$$C_{w,ss} = (k_u \times C_w) / (k_{ew} + g) \quad (6)$$

where  $C_{w,ss}$  is the metal concentration in mussels at steady state obtained from the dissolved phase ( $\mu\text{g g}^{-1}$ );

$$C_{f,ss} = \frac{\sum_{j=1}^n (AE \times IR \times C_w \times Kd)}{k_{ef} + g} \quad (7)$$

where  $C_{f,ss}$  is the metal concentration in mussels at steady state obtained from food ( $\mu\text{g g}^{-1}$ ). Thus, the relative importance of dissolved uptake and particulate ingestion can be calculated as:

$$R_w = C_{w,ss} / C_{ss} \quad (8)$$

$$R_f = C_{f,ss} / C_{ss} \quad (9)$$

where  $R_w$  is the proportion of accumulated trace element in a mussel obtained from the dissolved phase, and  $R_f$  is the proportion of accumulated trace element in a mussel obtained from food.

## RESULTS

### Trace element assimilation from ingested natural seston assemblages

The characteristics of the natural seston during the spring phytoplankton bloom in Long Island Sound are shown in Table 1. Seawater was sampled at high tide and low tide to produce 2 contrasting conditions of particle composition. Seston collected at high tide (March 4) consisted primarily of phytoplankton (>70% of particles), whereas seston collected at low tide (March 11) consisted mostly of inorganic particles (>70%). In both water samples, the phytoplankton assemblage was dominated by the diatom *Skeletonema costatum*, with chl *a* concentrations ranging from 11.7 to 17.2  $\mu\text{g l}^{-1}$ . At the end of the radiolabeling period, 32% of  $^{110m}\text{Ag}$ , 76% of  $^{241}\text{Am}$ , 3% of  $^{109}\text{Cd}$ ,

Table 1. Characteristics of natural seston collected from Stony Brook Harbor during the spring phytoplankton bloom in Long Island Sound (New York, USA) in 1995. Sample weights are on dry wt basis

	March 4 (high tide)	March 11 (low tide)
Total suspended solid (TSS, $\text{mg l}^{-1}$ )	4	9.5
Chl <i>a</i> ( $\mu\text{g l}^{-1}$ )	17.2	11.7
Chl <i>a</i> ( $\mu\text{g mg}^{-1}$ TSS)	4.3	1.2
Phytoplankton abundance	>70%	<30%
Algal composition	<i>Skeletonema costatum</i> (ca 70%) <i>Thalassiosira</i> sp. <i>Asterionella</i> sp. <i>Thalassionema</i> sp. <i>Coscinodiscus</i> sp.	<i>Skeletonema costatum</i> (60%) <i>Thalassiosira</i> sp. <i>Asterionella</i> sp. <i>Thalassionema</i> sp. <i>Chaetoceros</i> sp. <i>Nitzschia</i> sp.
Labeling conditions		
TSS ( $\text{mg l}^{-1}$ )	220	360
Chl <i>a</i> ( $\mu\text{g l}^{-1}$ )	488	328
Chl <i>a</i> ( $\mu\text{g mg}^{-1}$ TSS)	2.22	0.91

40% of  $^{57}\text{Co}$ , 3% of  $^{75}\text{Se}$ , and 11% of  $^{65}\text{Zn}$  were associated with the seston.

Depuration of the 6 radioisotopes in mussels following pulse radioactive feeding is shown in Fig. 1. Typically,  $^{110m}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{109}\text{Cd}$ ,  $^{75}\text{Se}$  and  $^{65}\text{Zn}$  were rapidly egested within the first 24 h, after which very little was lost from the mussels. The depuration of  $^{57}\text{Co}$ , however, was characterized by an initial rapid egestion within the first 12 h and then a slower steady egestion. Assimilation efficiencies (AEs) were calculated as the amount of radioactivity retained at 72 h divided by the amounts of radioactivity ingested (measured after the 0.5 h pulse radioactive feeding) (Table 2). AEs were highest for Se and Zn (28 to 41%), and were  $\leq 20\%$  for the other elements. Among the 6 trace elements,  $^{241}\text{Am}$  was the least available element to the mussels, with an AE <1%. AEs measured for the 2 seston assemblages were not significantly different for  $^{241}\text{Am}$ ,  $^{57}\text{Co}$ ,  $^{75}\text{Se}$  and  $^{65}\text{Zn}$ , but were different for  $^{110m}\text{Ag}$  and  $^{109}\text{Cd}$  ( $p < 0.05$ ).  $^{110m}\text{Ag}$  was more efficiently assimilated by mussels when more phytoplankton was present in seston, especially during the second phase of digestion. By contrast,  $^{109}\text{Cd}$  was more efficiently assimilated when less chl *a* was present in the seston.

AEs were also related to the fractions of radioisotopes in the cytoplasm of phytoplankton cells; there was an approximate 1.1 relationship between AE and cytosolic distribution for Am, Co and Se (Fig. 2). Generally, the greater assimilation of Se and Zn were related to a higher percentage of these elements in the cytoplasm of the seston. Less Cd was assimilated than would be expected from its cytosolic partitioning.

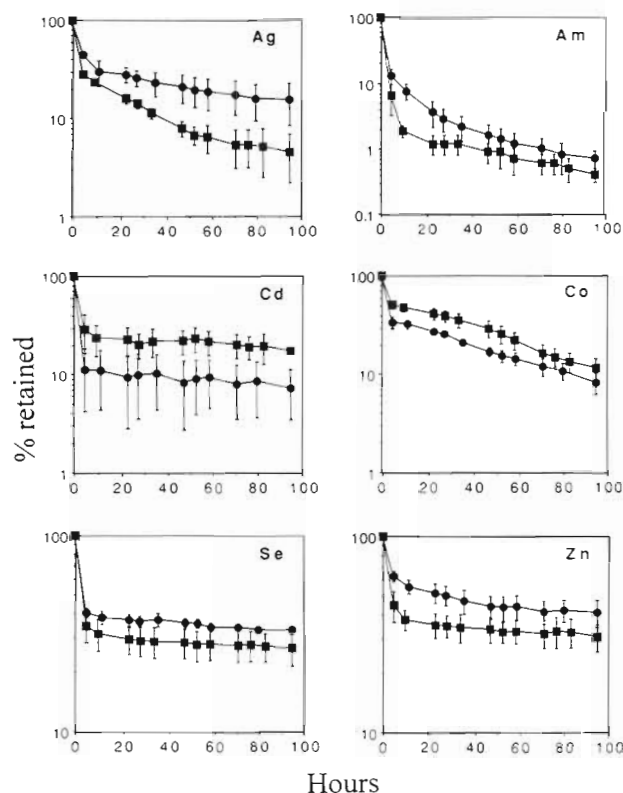


Fig. 1. *Mytilus edulis*. Retention of 6 trace elements in whole mussels following ingestion of radiolabeled natural seston. (●) Natural seston with chl *a* concentration of  $17.2 \mu\text{g l}^{-1}$ ; (■) natural seston with chl *a* concentration of  $11.7 \mu\text{g l}^{-1}$ . Means  $\pm$  SD ( $n = 5$ )

#### Uptake of trace elements from the dissolved phase

A statistically significant ( $p < 0.001$ ) linear relationship was observed between influx rates of the trace elements from the dissolved phase and the element concentrations in the dissolved phase (Fig. 3, Table 3). The coefficients of this relationship (log-log) for all elements were close to 1, indicating that uptake proceeded by adsorption and conformed with Freundlich absorption isotherms. Replicate experiments conducted in different years and seasons were directly comparable. The calculated dissolved uptake rate constant (Eq. 3, reflecting differences in efficiency of uptake) was greatest for Ag, followed by  $\text{Zn} > \text{Am} \approx \text{Cd} > \text{Co} > \text{Se}$  (Table 3). In these experiments, the filtration rates of mussels were not quantified. To calculate the absorption efficiency of trace elements from the dissolved phase (Table 3), the filtration rate of mussels was assumed to be  $117 \text{ l g}^{-1} \text{ d}^{-1}$  (mean from 26 locations; Widdows et al. 1995). Consequently, the estimated mean absorption efficiencies were 1.53% for Ag, 0.34% for Am, 0.31% for Cd, 0.11% for Co, 0.03% for Se, and 0.89% for Zn.

Table 2. Assimilation efficiencies (AEs) of 6 trace elements in mussels ingesting radiolabeled natural seston. Data are means  $\pm$  SD,  $n = 5$

Element	March 4 Chl <i>a</i> , $17.2 \mu\text{g l}^{-1}$	March 11 Chl <i>a</i> , $11.7 \mu\text{g l}^{-1}$
$^{109}\text{Cd}$	$17.6 \pm 6.8$	$5.4 \pm 2.3$
$^{241}\text{Am}$	$1.0 \pm 0.4$	$0.6 \pm 0.2$
$^{109}\text{Cd}$	$8.0 \pm 4.5$	$20.1 \pm 5.6$
$^{57}\text{Co}$	$11.9 \pm 2.5$	$16.2 \pm 3.6$
$^{75}\text{Se}$	$33.9 \pm 1.2$	$27.5 \pm 4.8$
$^{65}\text{Zn}$	$41.1 \pm 5.6$	$31.9 \pm 5.1$

The influx rate of trace elements from the dissolved phase increased as the salinity decreased from 34 to 20‰ (Fig. 4). At 20‰, the influx rate increased by 1.6 to 1.9 times for Ag, Cd, Co, Se and Zn compared to their respective influx rates at 34‰, whereas for Am the influx rate was increased by 2.5 times. Between 15 and 20‰, however, influx rates for all 6 trace elements remained relatively constant.

The DOM concentrations in Stony Brook (SB) and Southampton (SA) seawater were  $2.65 \pm 0.05$  and  $1.50 \pm 0.02 \text{ mg l}^{-1}$ , respectively. UV photooxidation removed about 50% of the DOM, with remaining concentrations of  $1.30 \pm 0.03$  and  $0.74 \pm 0.01 \text{ mg l}^{-1}$  for SB and SA seawater, respectively. Generally, no statistically significant difference in trace element influx rate was detected among different treatments (water batches or DOM levels), although in most cases trace elements in UV-irradiated seawater were accumulated at a slightly greater rate than in unirradiated seawater (Fig. 5).  $^{241}\text{Am}$  was accumulated at a rate  $>1.5$  times

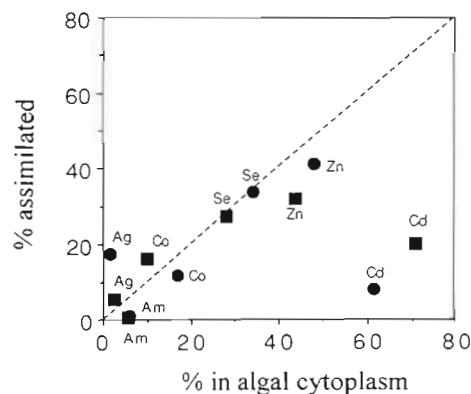


Fig. 2. *Mytilus edulis*. Relationship of assimilation efficiencies (AE) of trace elements in mussels and their cytoplasmic distributions in natural phytoplankton assemblages used as food. (●) Natural seston with chl *a* concentration of  $17.2 \mu\text{g l}^{-1}$ ; (■) natural seston with chl *a* concentration of  $11.7 \mu\text{g l}^{-1}$ . Data points are means of 5 individual mussels. Dotted line indicates a 1:1 relationship



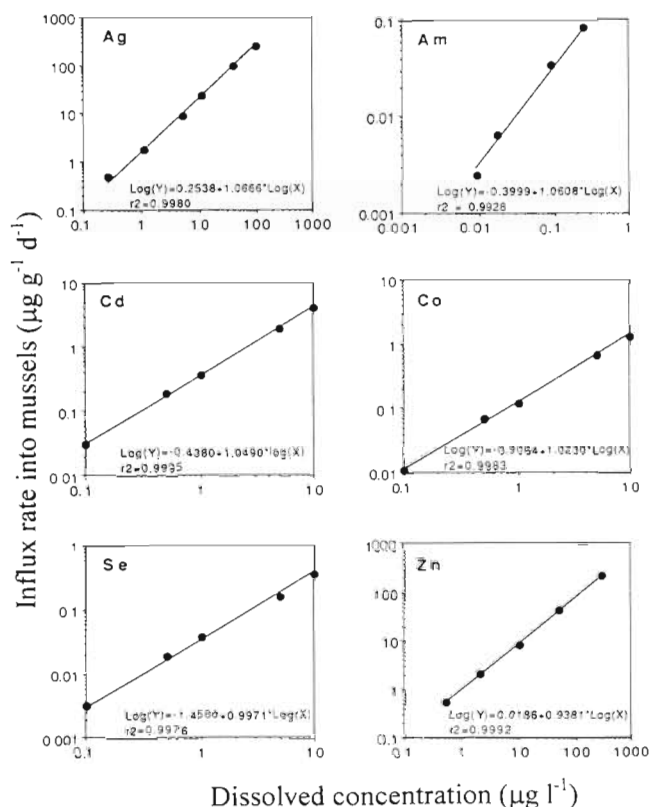


Fig. 3. *Mytilus edulis*. Influx rates ( $\mu\text{g g}^{-1} \text{dry wt d}^{-1}$ ) of trace elements into mussels from the dissolved phase at different dissolved trace element concentrations. Means  $\pm$  SD ( $n = 8$ ). The standard deviations are generally smaller than the symbols

higher in UV-oxidized SB water than in unirradiated SB water.

#### Efflux rates of trace elements

Rates of efflux from the most important physiological compartments in the mussels were usually similar whether food or the dissolved phase was the source of

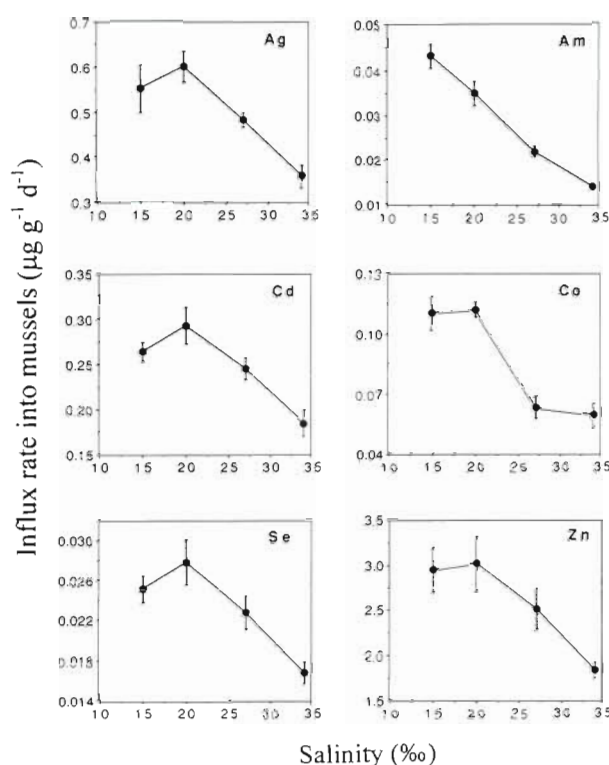


Fig. 4. *Mytilus edulis*. Influx rates ( $\mu\text{g g}^{-1} \text{dry wt d}^{-1}$ ) of trace elements into mussels from the dissolved phase at different salinities. Means  $\pm$  1 SE ( $n = 8$ )

exposure. Retention of the 6 radiotracers following 7 d ingestion of radiolabeled food is shown in Fig. 6. Depuration patterns could be described by 1-compartmental ( $^{109}\text{Cd}$ ), 2-compartmental ( $^{75}\text{Se}$ ), or 3-compartmental ( $^{110\text{m}}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{57}\text{Co}$ , and  $^{65}\text{Zn}$ ) first-order exponential loss patterns. Physiological rate constants of loss were calculated from the slowest compartment of loss. The rate constants for this compartment ranged from 0.98 %  $\text{d}^{-1}$  for  $^{57}\text{Co}$  to 3.4 %  $\text{d}^{-1}$  for  $^{110\text{m}}\text{Ag}$  [Table 4 also includes the biological half-life ( $t_{1/2}$ ) of each compartment]. The contribution of the slowest compartment ranged from 12 % in Ag to 95 % in Cd. The most rapid

Table 3. Relationships describing trace element influx rate ( $I_u$ ,  $\mu\text{g g}^{-1} \text{d}^{-1}$ ) in mussels and dissolved trace element concentration ( $C_w$ ,  $\mu\text{g l}^{-1}$ ) in seawater. The ranges of dissolved uptake rate constants ( $k_u$ ), calculated using regression of log-transformed influx rates and dissolved concentrations, are also shown. Absorption efficiencies were calculated by dividing  $k_u$  by the mussel filtration rate (mean  $117 \text{ l g}^{-1} \text{d}^{-1}$ ; range 52 to  $196 \text{ l g}^{-1} \text{d}^{-1}$ ), from Widdows et al. (1995). Sample weights are on dry wt basis

Element	Equation	$r^2$	$k_u$ range ( $\text{l g}^{-1} \text{d}^{-1}$ )	Absorption efficiency (%) Mean	Range
Ag	$I_u = 1.794 [C_w]^{0.066 \pm 0.0024 \text{ (SE)}}$	0.998	1.595–2.018	1.53	0.92–3.45
Am	$I_u = 0.398 [C_w]^{1.061 \pm 0.064 \text{ (SE)}}$	0.992	0.337–0.470	0.34	0.20–0.76
Cd	$I_u = 0.365 [C_w]^{1.049 \pm 0.094 \text{ (SE)}}$	0.999	0.346–0.384	0.31	0.19–0.70
Co	$I_u = 0.124 [C_w]^{1.023 \pm 0.024 \text{ (SE)}}$	0.998	0.113–0.136	0.11	0.06–0.23
Se	$I_u = 0.035 [C_w]^{0.997 \pm 0.028 \text{ (SE)}}$	0.998	0.032–0.039	0.03	0.02–0.07
Zn	$I_u = 1.044 [C_w]^{0.969 \pm 0.016 \text{ (SE)}}$	0.999	0.963–1.131	0.89	0.53–2.01



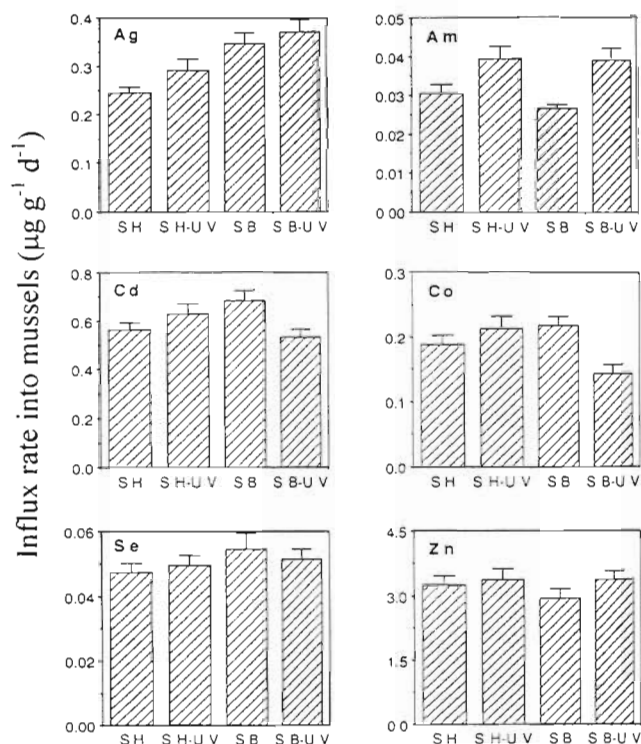


Fig. 5. *Mytilus edulis*. The influx rates ( $\mu\text{g g}^{-1}$  dry wt  $\text{d}^{-1}$ ) of trace elements into mussels from the dissolved phase using different batches of seawater with and without UV photooxidation. Means  $\pm 1$  SE ( $n = 8$ ). SH: Southampton seawater, not UV-oxidized; SH-UV: Southampton seawater, UV-oxidized; SB: Stony Brook Harbor seawater, not UV-oxidized; SB-UV: Stony Brook Harbor seawater, UV-oxidized

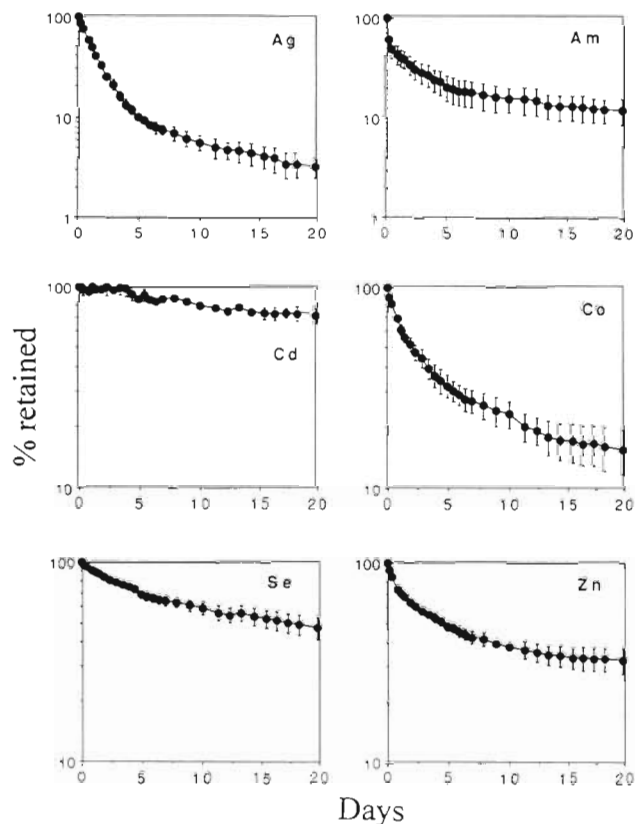


Fig. 6. *Mytilus edulis*. The retention of 6 trace elements in whole mussels (soft parts and shell) following 7 d ingestion of the radiolabeled diatom *Thalassiosira pseudonana*. Means  $\pm$  SD ( $n = 5$ )

Table 4. *Mytilus edulis*. Compartmental analysis of trace element depuration in whole mussels following 7 d ingestion of radiolabeled diatom cells *Thalassiosira pseudonana*. Each compartment assumed a first order depuration, conforming to  $A = A_0 \times \exp(-k \times t)$ , where  $A$  is the % retained at time  $t$ ,  $A_0$  is the initial % retained, and  $k$  is the rate constant. The biological half-life ( $tb_{1/2}$ , d) of each compartment is also calculated. The depuration rate constant measured in the slowest compartment is representative of the physiological efflux of the trace element. Data are means  $\pm$  SD,  $n = 5$

Element	Period (d)	% in compartment	$k$ ( $\% \text{ d}^{-1}$ )	$tb_{1/2}$ (d)	$r^2$
Ag	0–4.8	$81.2 \pm 2.8$	$34.1 \pm 3.5$	$2.1 \pm 0.2$	0.970
	4.8–7.8	$7.0 \pm 3.9$	$5.1 \pm 3.2$	$22.7 \pm 17.8$	0.900
	7.8–20	$11.8 \pm 1.1$	$3.4 \pm 0.8$	$22.0 \pm 5.6$	0.876
Am	0–0.4	$47.9 \pm 9.2$	$154.0 \pm 4.7$	$0.5 \pm 0.1$	0.905
	0.4–4.8	$23.0 \pm 7.9$	$11.4 \pm 3.4$	$7.0 \pm 3.0$	0.960
	4.8–20	$29.1 \pm 6.9$	$2.0 \pm 0.3$	$35.4 \pm 5.5$	0.947
Cd	5–20	$95.4 \pm 6.3$	$1.4 \pm 0.7$	$67.4 \pm 39.5$	0.714
Co	0–3.8	$49.7 \pm 5.9$	$15.5 \pm 2.5$	$4.6 \pm 0.8$	0.939
	3.8–12.3	$17.2 \pm 5.7$	$3.2 \pm 0.8$	$23.6 \pm 7.2$	0.929
	12.3–20	$33.0 \pm 0.6$	$0.98 \pm 0.16$	$72.3 \pm 10.6$	0.868
Se	0–6.3	$24.6 \pm 5.8$	$4.2 \pm 1.1$	$18.5 \pm 6.8$	0.983
	6.3–20	$75.4 \pm 5.8$	$2.2 \pm 0.8$	$39.1 \pm 21.4$	0.921
Zn	0–1.4	$29.5 \pm 3.6$	$20.9 \pm 2.1$	$3.3 \pm 0.4$	0.967
	1.4–8.8	$25.8 \pm 5.7$	$5.1 \pm 1.3$	$14.7 \pm 4.8$	0.967
	8.8–20	$44.7 \pm 2.2$	$1.5 \pm 1.0$	$66.5 \pm 39.0$	0.737

phase of efflux resulted from defecation of ingested radiolabeled material that was not assimilated. Thus, this compartment was largest for those elements least efficiently assimilated ( $>73\%$  for  $^{110m}\text{Ag}$ ,  $^{241}\text{Am}$  and  $^{57}\text{Co}$ ) and was very small for  $^{109}\text{Cd}$  (Table 4). This loss was not indicative of physiological efflux.

Depuration patterns following 12 h or 6 d uptake from the dissolved phase were typically characterized by at least a 2-compartmental loss pattern (Fig. 7, Table 5). Efflux rate constants calculated from the slowest compartment after 6 d uptake from the dissolved phase ranged from  $1.1\% \text{ d}^{-1}$  for  $^{109}\text{Cd}$  to  $2.6\% \text{ d}^{-1}$  for  $^{75}\text{Se}$  (Table 5). With the exception of  $^{110m}\text{Ag}$ , these rates were not significantly different from efflux rate constants measured after 7 d of food ingestion ( $p > 0.05$ ). In addition, there were no significant differences in efflux rate constants observed between the 12 h and 6 d exposures ( $p > 0.05$ ), suggesting that the 12 h exposure was sufficient for the radioisotopes to be partitioned into the slowest compartment. However, the proportion of total radioisotope body burden in the slowest compartment was smaller with the shorter dissolved exposure (Fig. 7,

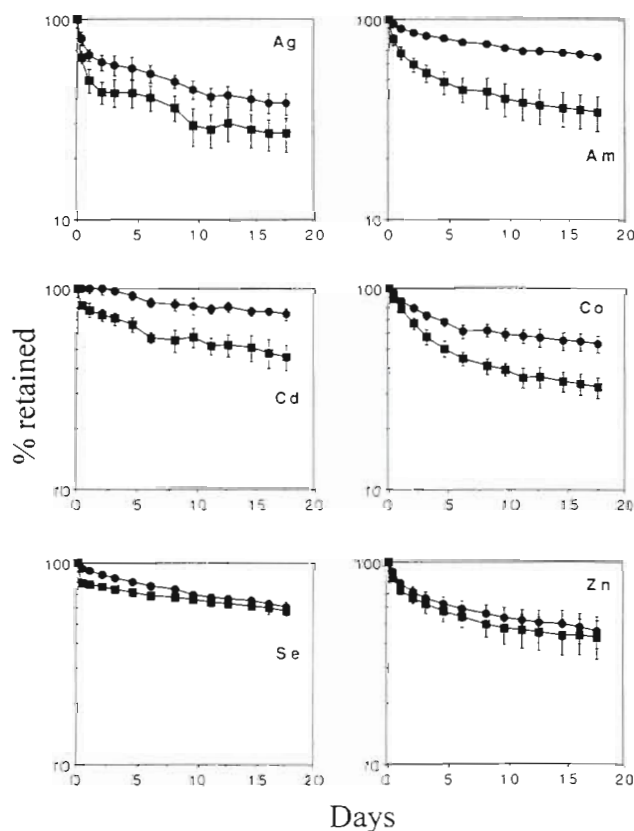


Fig. 7 *Mytilus edulis*. Retention of 6 trace elements in whole mussels (soft parts and shell) following 12 h or 6 d of exposure to the elements in the dissolved phase. (■) 12 h exposure; (●) 6 d exposure. Means  $\pm$  SD ( $n = 6$  to 8)

Table 5). The greatest loss rates occurred within the first 24 h of depuration, presumably due to rapid desorption and possibly defecation.

After 7 d of radioactive feeding, substantial fractions (10 to 30%) of  $^{110m}\text{Ag}$ ,  $^{241}\text{Am}$ , and  $^{57}\text{Co}$  were found on the shells, whereas most of  $^{109}\text{Cd}$ ,  $^{75}\text{Se}$ , and  $^{65}\text{Zn}$  was associated with the digestive glands and soft tissues (Fig. 8). In the experiments assessing radioisotope uptake from the dissolved phase, it is apparent from the tissue distributions of the radioisotopes that, with the exception of  $^{109}\text{Cd}$ , the shells contained much of the accumulated radioisotopes (Fig. 9).

### Kinetic modeling of trace element accumulation in mussels

The kinetic model described in Eq. (2) requires measurements of trace element AE in the mussel, influx rate constant from the dissolved phase ( $k_u$ ), efflux rates ( $k_e$ ), mussel ingestion rate (IR), the dissolved concentration ( $C_w$ ), and partition coefficients ( $K_d$ ) for sus-

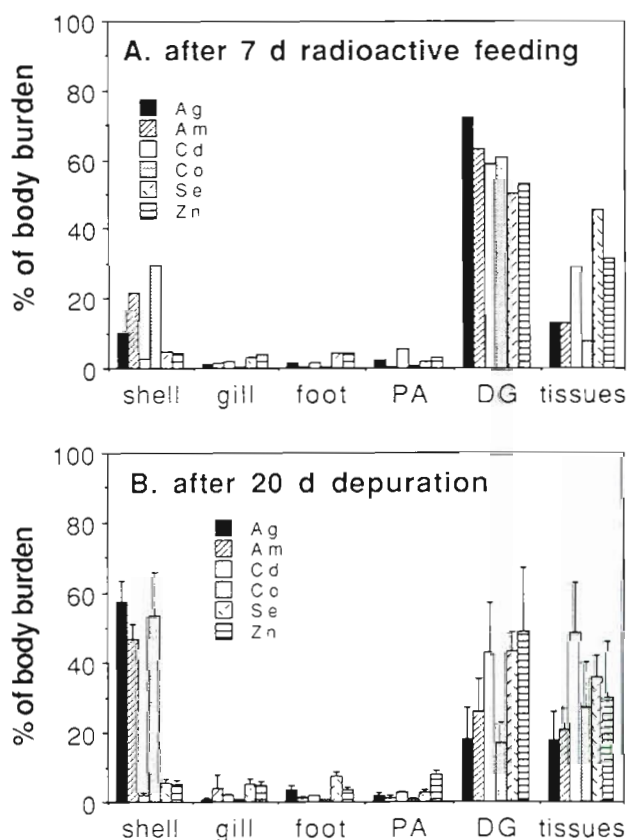


Fig. 8 *Mytilus edulis*. Distribution of 6 radiotracers in mussel tissues (A) after 7 d ingestion of the radiolabeled diatom *Thalassiosira pseudonana* and (B) after 20 d of depuration. Means  $\pm$  SD ( $n = 2$ ). PA: posterior adductor; DG: digestive gland

Table 5. *Mytilus edulis*. Compartmental analysis of trace element depuration in whole mussels following 12 h and 6 d dissolved uptake, respectively. Each compartment assumed a first order depuration, conforming to  $A = A_0 \times \exp(-k \times t)$ , where  $A$  is the % retained at time  $t$ ,  $A_0$  is the initial % retained, and  $k$  is the rate constant. The depuration rate constant measured in the slowest compartment is representative of the physiological efflux of trace element. The biological half-life ( $tb_{1/2}$ ) of each compartment is also shown. Data are means  $\pm$  SD,  $n = 6$  to 8

Element		Period (d)	% in compartment	k (% , d <sup>-1</sup> )	tb <sub>1/2</sub> (d)	r <sup>2</sup>	
Ag	12 h	0-1	49.8 ± 6.4	62.5 ± 12.1	1.2 ± 0.3	0.893	
		1-9.5	21.8 ± 13.1	3.6 ± 1.4	23.7 ± 12.1	0.789	
		9.5-18	35.4 ± 7.8	1.5 ± 0.4	49.5 ± 17.8	0.638	
	6 d	0-1	33.9 ± 6.2	36.1 ± 5.8	2.0 ± 0.3	0.932	
		1-9.5	13.0 ± 2.5	1.9 ± 0.5	38.6 ± 9.5	0.882	
		9.5-18	51.8 ± 4.8	1.9 ± 0.3	37.9 ± 7.6	0.832	
Am	12 h	0-1	32.9 ± 4.5	30.2 ± 5.7	2.4 ± 0.5	0.915	
		1-6	18.3 ± 5.1	5.0 ± 1.8	16.1 ± 6.6	0.983	
		6-18	50.0 ± 8.7	2.3 ± 0.3	30.9 ± 4.2	0.924	
	6 d	0-2	13.8 ± 2.5	5.3 ± 0.9	13.4 ± 2.2	0.902	
		2-18	86.2 ± 2.5	1.9 ± 0.5	39.0 ± 8.4	0.937	
Cd	12 h	0-0.3	16.0 ± 7.3	51.5 ± 14.1	1.4 ± 0.3	1.000	
		0.3-6	16.2 ± 6.4	3.8 ± 1.3	22.3 ± 12.3	0.920	
		6-18	67.1 ± 5.0	2.1 ± 0.6	34.0 ± 7.6	0.760	
	6 d	6-18	90.7 ± 7.6	1.1 ± 0.4	78.3 ± 39.9	0.693	
Co	12 h	0-3	35.8 ± 6.6	12.6 ± 3.6	6.0 ± 1.5	0.982	
		3-11	16.0 ± 4.9	3.1 ± 0.8	23.7 ± 4.9	0.927	
		11-18	48.2 ± 5.9	2.3 ± 0.4	30.9 ± 5.3	0.981	
	6 d	0-1	10.1 ± 4.7	8.3 ± 3.4	9.8 ± 3.5	0.978	
		1-6	17.7 ± 5.2	3.9 ± 0.9	19.0 ± 5.7	0.986	
		6-18	69.8 ± 7.4	1.8 ± 0.7	45.2 ± 19.2	0.879	
Se	12 h	0-0.3	21.9 ± 2.0	66.3 ± 13.9	1.1 ± 0.3	1.000	
		0.3-18	78.1 ± 2.0	1.9 ± 0.5	40.2 ± 12.6	0.928	
	6 d	0-0.3	9.1 ± 4.0	16.7 ± 4.5	4.4 ± 1.2	1.000	
		0.3-18	90.9 ± 4.0	2.6 ± 0.6	28.1 ± 6.0	0.972	
Zn	12 h	0-1	28.2 ± 5.5	25.4 ± 5.7	2.9 ± 0.6	0.899	
		1-8	17.9 ± 6.7	3.4 ± 1.1	22.7 ± 8.3	0.944	
		8-18	54.0 ± 6.3	1.5 ± 0.7	53.2 ± 19.6	0.799	
	6 d	0-1	23.7 ± 4.2	18.7 ± 3.8	3.8 ± 0.7	0.973	
		1-8	13.1 ± 3.1	2.3 ± 0.6	31.6 ± 8.0	0.968	
		8-18	63.8 ± 4.0	2.0 ± 0.9	43.6 ± 18.9	0.906	

pended particles (if direct trace element concentrations on these particles are not known). Model predictions for a specific environment require reliable data for trace element concentrations in seawater and in seston; field verification can be accomplished by concurrent analyses of mussel tissues.

Bayne et al. (1987, 1993) measured mussel ingestion rates on seston prepared from mixtures of silt and algae with a wide range of organic content (9 to 72%). These studies showed that mussels regulated their feeding activity in response to short-term changes in their diets and were able to maintain ingestion rates in a simple relationship with the seston concentration (Bayne 1993). Calculations using Bayne et al.'s (1987, 1993) data gave the following relationship between ingestion rate of seston, IR (mg g<sup>-1</sup> d<sup>-1</sup>) and total seston concentration (TSS, mg l<sup>-1</sup>):

$$IR = 137 [TSS]^{0.4212} \quad (r^2 = 0.573) \quad (10)$$

Mussels produce pseudofeces at high seston concentrations ( $\geq 5$  mg l<sup>-1</sup>; Widdows et al. 1979) and maintain

a maximum ingestion rate beyond this level. Thus, at seston concentrations  $\geq 5$  mg l<sup>-1</sup>, the mussels were assumed to maintain a maximum ingestion rate of 270 mg g<sup>-1</sup> d<sup>-1</sup>. The growth rate constant (0.0019 d<sup>-1</sup>; Thomann 1981, Connolly 1991) is 1 order of magnitude lower than the efflux rate constant measured in this study. Therefore, the growth rate constant was ignored in our modeling calculations.

Table 6 summarizes the biological parameters for each trace element employed in the bioaccumulation modeling. AEs were taken from previous studies (Wang & Fisher 1996a, b) and this study in order to take into account a diversity of feeding strategies. AEs of trace elements in mussels decrease slightly with increasing amounts of food material they ingest (Wang et al. 1995). Since suspended particle loads are relatively high in mussel beds and shallow coastal waters, a lower range of AEs observed for diverse phytoplankton cells (Wang & Fisher 1996a) and natural seston (Table 2) was employed in our modeling.

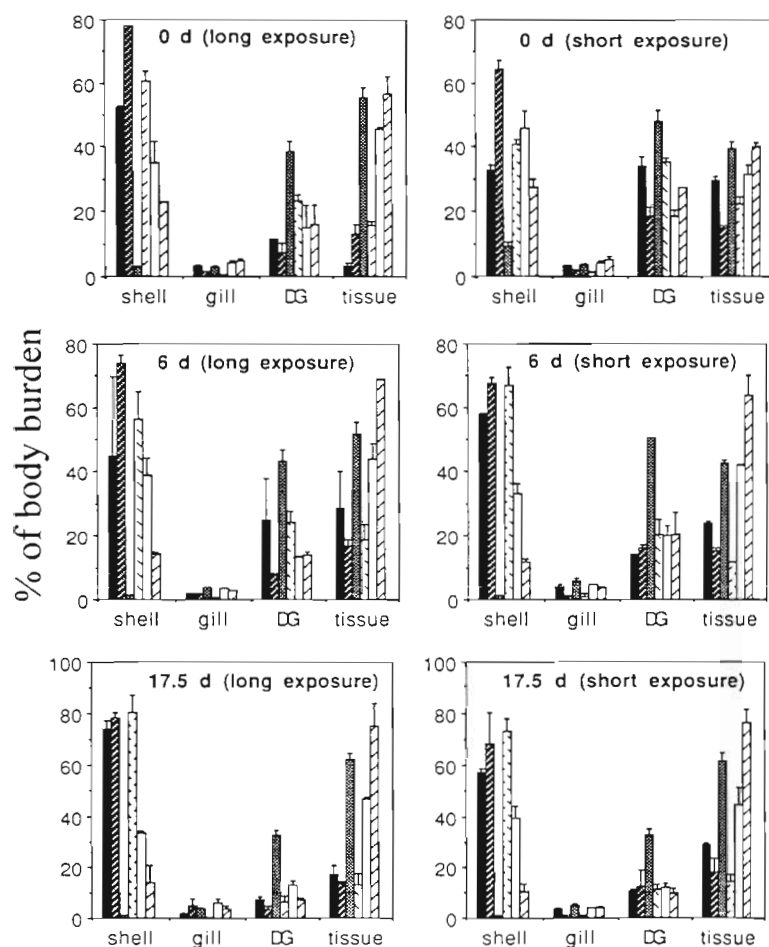


Fig. 9. *Mytilus edulis*. Distribution of 6 radiotracers in mussel tissues following short-term (12 h) or long-term (6 d) exposure to the elements in the dissolved phase, and during the 17.5 d depuration period. Means  $\pm$  SD ( $n = 2$ ). DG: digestive gland. For each tissue, bars are (from left to right) Ag, Am, Cd, Co, Se, Zn

The concentrations and partitioning of trace elements in San Francisco Bay (SFB) have been extensively studied using trace element clean techniques (Cutter 1989, Kuwabara et al. 1989, Flegal et al. 1991,

Table 6. Trace element parameters used in the bioaccumulation modeling.  $k_u$ : uptake rate constant from the dissolved phase; AE: assimilation efficiency;  $k_{el}$ : efflux rate constant after food uptake;  $k_{ew}$ : efflux rate constant after dissolved uptake. AE values from Wang & Fisher (1996a)

Element	$k_u$ ( $l\ g^{-1}\ d^{-1}$ )	AE (%)	$k_{el}$ ( $d^{-1}$ )	$k_{ew}$ ( $d^{-1}$ )
Ag	1.794	4–34	0.034	0.019
Am	0.398	1–6	0.020	0.019
Cd	0.365	11–40	0.014	0.011
Co	0.124	20–43	0.0098	0.018
Se	0.035	15–72	0.022	0.026
Zn	1.044	16–48	0.015	0.020

Smith & Flegal 1993, Wood et al. 1995). Below, we use the low and high dissolved concentrations measured at Dumbarton Bridge Station to model trace element concentrations in mussels and compare these with NS&T measurements of trace elements in mussels at this station (South SFB). Seston concentrations at this station were typically  $>10\ mg\ l^{-1}$ , therefore a maximum mussel ingestion rate ( $270\ mg\ g^{-1}\ d^{-1}$ , calculated from Eq. 10) was assumed. The partition coefficients for Ag, Cd, Se and Zn in SFB are shown in Table 7. Partition coefficients for each element are dependent on environmental conditions, particularly the composition of the seston and possibly salinity. The  $K_d$  for Cd given in Kuwabara et al. (1989) was multiplied by a factor of 2, because their measurements of dissolved Cd concentrations were underestimated by a factor of 2 (Wood et al. 1995). An average  $K_d$  for each trace element measured in SFB was employed. Selenium speciates in seawater as selenite, selenate, elemental selenium, and organic selenides (Cutter & Bruland 1984), but we only modeled selenite bioaccumulation from our experiments which used dissolved selenite uptake. The selenite concentration factor in phytoplankton based on studies with selenite (geometric mean of  $2.8 \times 10^4$ ; Fisher & Reinfelder 1995) is generally higher than values calculated with field measurements, which are based on living and abiotic suspended particles and total dissolved selenium concentra-

tion. We therefore chose a  $K_d$  value of  $1 \times 10^4$  for selenite in our modeling

For comparative purposes we also modeled bioaccumulation in mussels in Long Island Sound (LIS). We calculated the likely dissolved concentrations in LIS based on the measurements of Hunt (1979) of trace metal concentrations in net phytoplankton collected from LIS in 1976, using geometric mean phytoplankton concentration factors (Fisher & Reinfelder 1995). Trace metal concentrations predicted in mussel tissues in LIS were compared with LIS data for mussel soft parts (Goldberg et al. 1983) measured at the same time as Hunt's (1979) study.

Under different environmental conditions likely to be encountered by the mussels in SFB and LIS, the trace element concentrations predicted from the independent laboratory experiments for mussel tissues using Eq. (5) were strikingly close to the actual tissue concentrations measured in the monitoring programs



Table 7 Partition coefficient ( $K_d$ , l kg dry wt<sup>-1</sup>) of trace elements in seston measured in South San Francisco Bay (California, USA). Am and Co  $K_d$ s in coastal sediments and phytoplankton are taken from IAEA (1985) and Fisher & Reinfelder (1995) (dry wt basis using a mean vol:dry wt ratio of 4.75; Fisher et al. 1983a)

Element	$K_d$	Mean $K_d$	Source
Ag	$6.3 \times 10^4 - 4.0 \times 10^5$	$1.6 \times 10^5$	Smith & Flegal (1993)
Cd	$4 \times 10^3 - 6 \times 10^3$	$5 \times 10^3$	Kuwabara et al. (1989)
Se	$2 \times 10^3 - 6 \times 10^3$	$4 \times 10^3$	Cutter (1989)
Zn	$1.6 \times 10^4 - 2.2 \times 10^4$	$1.9 \times 10^4$	Kuwabara et al. (1989)
Am	$10^5 - 2 \times 10^7$ (coastal sediments)	$2 \times 10^6$	IAEA (1985)
	Phytoplankton	$7.6 \times 10^5$	Fisher & Reinfelder (1995)
Co	$2 \times 10^4 - 10^6$ (coastal sediments)	$2 \times 10^5$	IAEA (1985)
	Phytoplankton	$2.1 \times 10^3$	Fisher & Reinfelder (1995)

(Table 8). For Cd and Se in both SFB and LIS, and Zn in LIS, the predicted concentrations were within the ranges of actual measured concentrations in mussels. For Ag in both SFB and LIS and Zn in SFB, the highest

predicted metal concentrations in mussel tissues using the highest dissolved concentrations and the high AEs were 2 to 3× higher than the metal concentrations measured in the field.

Table 8. *Mytilus edulis*. Model-predicted trace element concentration in mussel tissues ( $C_{ss}$ ) and its comparison with field-measured concentration ( $C_{measured}$ ) in mussels collected from Dumbarton Bridge, South San Francisco Bay (data from NS&T Program, T. P. O'Connor pers. comm.) and Long Island Sound (data from Goldberg et al. 1983). Seston concentrations at these stations were  $>7$  mg l<sup>-1</sup>, thus a maximum ingestion rate of 270 mg g<sup>-1</sup> d<sup>-1</sup> was assumed for mussels (calculated from Eq. 10). Both the lowest and highest dissolved concentrations were included in modeling (Ag, Cd and Zn dissolved concentration in SFB were from Flegal et al. 1991; Se data was from Cutter 1989). Also shown are the proportion of total body burden of each metal predicted to come from the dissolved phase. See text for detailed discussion of parameters. Sample weights are on dry wt basis

Element	$C_w$ ( $\mu\text{g l}^{-1}$ )	$K_d$	AE	$C_{ss}$ ( $\mu\text{g g}^{-1}$ )	$C_{measured}$ ( $\mu\text{g g}^{-1}$ )	% dissolved
<b>San Francisco Bay</b>						
Ag	0.0026	150000	0.04	0.29	0.35–0.77	57
	0.0097	150000	0.04	1.15		59
	0.0026	150000	0.12	0.54		31
	0.0097	150000	0.12	2.09		33
Cd	0.07	5000	0.1	2.7	4.4–9.4	76
	0.17	5000	0.1	6.8		76
	0.07	5000	0.3	4.1		51
	0.17	5000	0.3	10.1		52
Se	0.025	10000	0.3	1.0	2.5–6.7	3.6
(selenite)	0.065	10000	0.3	2.5		3.6
	0.025	10000	0.7	2.2		1.6
	0.065	10000	0.7	5.6		1.6
Zn	0.5	20000	0.15	54	54–130	52
	1.7	20000	0.15	176		50
	0.5	20000	0.3	80		35
	1.7	20000	0.3	265		33
<b>Long Island Sound</b>						
Ag	0.0038	150000	0.04	0.43	0.04–0.44	58
	0.0044	150000	0.04	0.51		58
	0.0038	150000	0.12	0.80		31
	0.0044	150000	0.12	0.93		31
Cd	0.074	5000	0.1	2.9	1.5–6.2	76
	0.120	5000	0.1	4.8		76
	0.074	5000	0.3	4.3		51
	0.120	5000	0.3	7.0		51
Zn	0.315	20000	0.15	34	52–142	52
	1.000	20000	0.15	105		51
	0.315	20000	0.3	51		36
	1.000	20000	0.3	157		34

### Determining the relative contributions of dissolved and particulate uptake routes

The proportion of the overall trace element accumulation in mussel tissues that is attributable to uptake from the dissolved phase was calculated at various dissolved concentrations with changing AE,  $K_d$ , and seston concentration. Because  $K_d$ 's were employed to determine  $C_i$  (Eq. 4), the relative importance of different uptake pathways is independent of  $C_w$  (see Eqs. 6 to 9) at steady state. Fig. 10 presents calculations for each trace element at various  $K_d$ , AE, and TSS values using assumptions described for the calculation of  $C_{ss}$ . More than 96% of Se bioaccumulation is calculated to be from food ingestion under various environmental conditions. For the other trace elements (Ag, Am, Cd, Co, Zn), the contribution of dissolved uptake depends on changes in environmental and physiological factors, decreasing with increasing  $K_d$  and with increasing AE. The total particle load has a relatively minor effect on the proportion of accumulated trace element from the dissolved phase, primarily because of the slightly inverse effect on AE and a constant ingestion rate at seston loads  $>5$   $\text{mg l}^{-1}$ . Under most of these conditions,  $>50\%$  of the Cd in mussels is from the dissolved phase, whereas  $>80\%$  of Am,  $>70\%$  of Co, and  $>50\%$  of Zn in mussels are directly from ingested particles. The pathways of Ag bioaccumulation differ most widely, with 30 to 70% coming from the dissolved phase.

### Factors affecting metal bioaccumulation in mussels

An analysis was also performed to quantify the influence of changes in  $C_i$ , TSS,  $K_d$ , salinity, and AE on trace element concentrations in mussel tissues. Because  $C_w$  (dissolved concentration,  $\mu\text{g l}^{-1}$ ) and  $C_i$  (particulate concentration,  $\mu\text{g mg}^{-1}$ ) are directly dependent on TSS,  $K_d$ , and the total concentration ( $C_t$ ,  $\mu\text{g l}^{-1}$ ) of trace element in the water column (that is, dissolved plus particulate trace element concentration), we calculated predicted values of  $C_w$  and  $C_i$  as a function of  $C_t$  at different TSS (total suspended solids) loads, assuming that  $K_d$  is independent of TSS loads (Fig. 11).

$$C_t = C_w + (C_i \times \text{TSS}) \quad (11)$$

By applying Eq. (4), Eq. (11) then becomes:

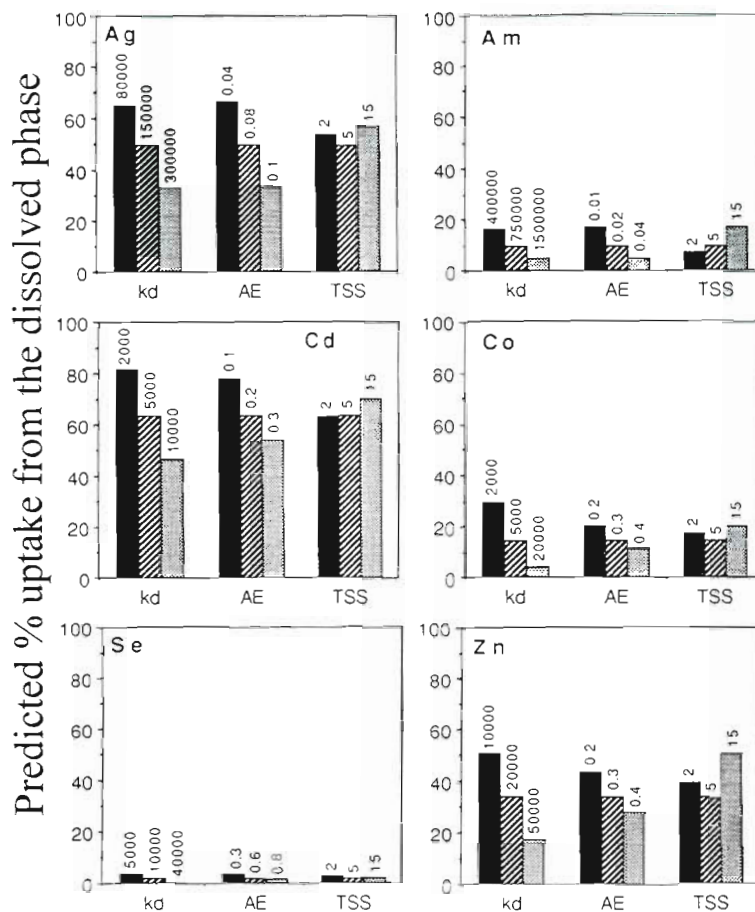


Fig. 10. *Mytilus edulis*. The calculated proportion (%) of the total trace element concentration in mussels from the dissolved phase over a range of different partition coefficients ( $K_d$ ), assimilation efficiencies (AE), and seston (TSS) loads (2 to 15  $\text{mg dry wt l}^{-1}$ ). The range of  $K_d$  and AE values used are specific to each element and are based on observed values (see also Tables 6 & 7). Calculations for each parameter (e.g.  $K_d$ ) assume intermediate values of the other 2 parameters (e.g. AE and TSS load) shown in the figure. See text for further explanation

$$\text{Thus,} \quad C_i = C_w + (C_w \times K_d \times \text{TSS}) \quad (12)$$

$$C_w = [1/(1 + \text{TSS} \times K_d)] \times C_i \quad (13)$$

Calculations using Eqs. (4) and (13) are shown in Fig. 11. Note that  $C_i$  is an independent variable and need not be constant. Both dissolved and particulate concentrations decline with increasing TSS loads, particularly for trace elements with  $K_d$  values  $>10^4$

Total metal concentration in the water column

Applying Eq. (13) to Eq. (5) it can be shown that:

$$C_{ss} = \left( \frac{k_u}{k_{ew} + g} + \frac{\text{AE} \times \text{IR} \times K_d}{k_{ef} + g} \right) \times \left( \frac{1}{1 + \text{TSS} \times K_d} \right) \times C_i \quad (14)$$

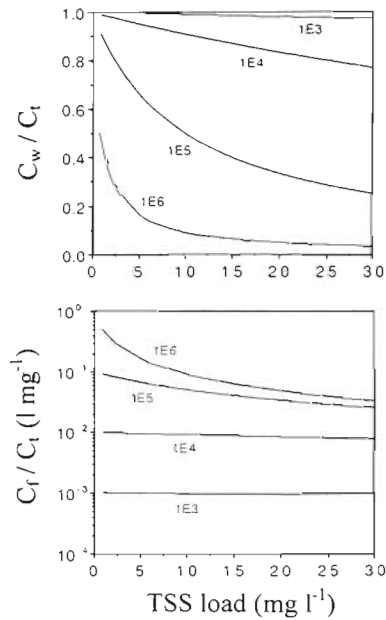


Fig. 11. Predicted range in the ratios of metal concentrations in the dissolved ( $C_w$ ) and particulate ( $C_i$ ) phases to total metal concentration ( $C_t$ ) at different total suspended solids (TSS) loads (1 to 30 mg dry wt  $l^{-1}$ ) and  $K_d$ s ( $10^3$  to  $10^6$ ). These calculations assume that  $K_d$ s are independent of the TSS load (Eqs. 4 & 13)

Eq. (14) conceptually illustrates the variety of factors that can affect bioaccumulation.  $C_{ss}$  is directly proportional to  $C_i$  under all conditions, consistent with the fundamental concept underlying the Mussel Watch Program. However, the environment and the organism can influence bioavailability, i.e. there is no constant proportionality between  $C_{ss}$  and  $C_i$ . Changes in any of the parameters described in Eq. (14) can greatly affect the ratio of  $C_{ss}$  to  $C_i$ , a mathematical expression of the widely employed bioaccumulation factor (BAF). The BAF as defined here thus considers both dissolved and particulate metal uptake in mussels.

$$BAF = \frac{C_{ss}}{C_i} = \left( \frac{k_{li}}{k_{ew} + g} + \frac{AE \times IR \times K_d}{k_{ef} + g} \right) \left( \frac{1}{1 + TSS \times K_d} \right) \quad (15)$$

#### Total suspended solids loads

TSS loads can affect several parameters in the bioaccumulation model, including  $C_w$  (Eq. 13), IR (Eq. 10) and AE. For all our calculations, IR was assumed to be 270 mg  $g^{-1} d^{-1}$  at TSS > 5 mg  $l^{-1}$ . AE was assumed to vary by a factor < 2 over a TSS range of 1 to 20 mg  $l^{-1}$ , with AEs increasing inversely with TSS loads (Wang et al. 1995). Using Eq. (15), BAF values were calculated for different TSS loads. The greatest effect of TSS load

on BAF was found for Ag and Am, both of which are particle-reactive metals with  $K_d$  values >  $10^5$  (Fig. 12). For these metals, with increasing TSS loads, metal concentrations in both dissolved and particulate phases decline appreciably, resulting in a precipitous decline in metal concentration in mussel tissues (> 3× for Ag and > 10× for Am). TSS loads have a much less pronounced effect on BAF for Cd and Co (Fig. 12), primarily due to their lower  $K_d$  values (about 5000). At the lower range of TSS loads, BAF increases with TSS load for Cd, Co, Se, and Zn due to a significant increase in IR and a relatively small effect of  $K_d$  on  $C_w$ . However, with increasing TSS loads (> 5 mg  $l^{-1}$ ), BAF declines due to a decrease in AE and  $C_w$ .

#### Assimilation efficiency

In contrast to the effects of TSS load, BAF values increase linearly with AE (Fig. 13). These calculations assumed a constant  $K_d$  for each element and a TSS load of 5 mg  $l^{-1}$ . Se is most affected by AE, with a slope of about 1 (calculated from the regression of change in AE with

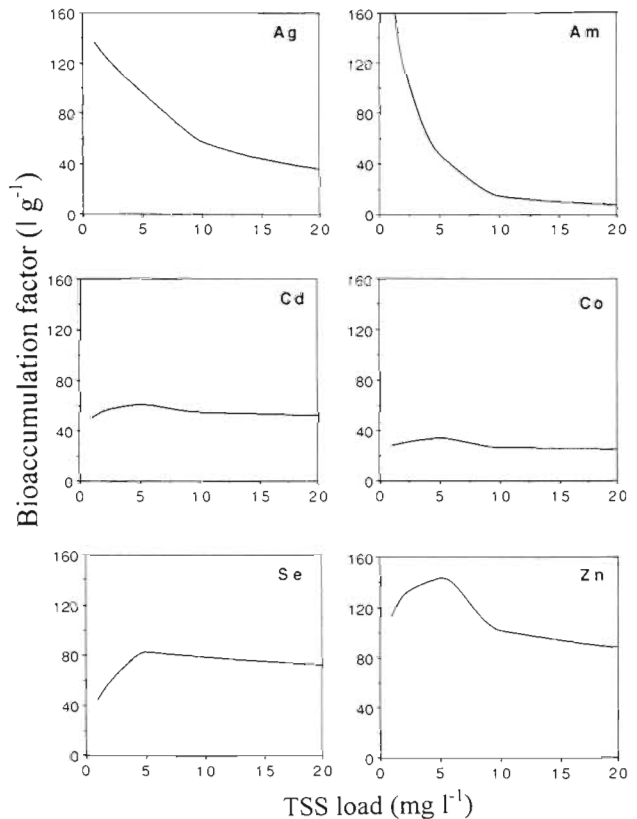


Fig. 12. *Mytilus edulis*. Predicted bioaccumulation factor (BAF,  $l g^{-1}$  dry wt) of 6 trace elements in mussel soft parts at different TSS loads, over a range of 1 to 20 mg dry wt  $l^{-1}$ . BAF calculated using Eq. (15)

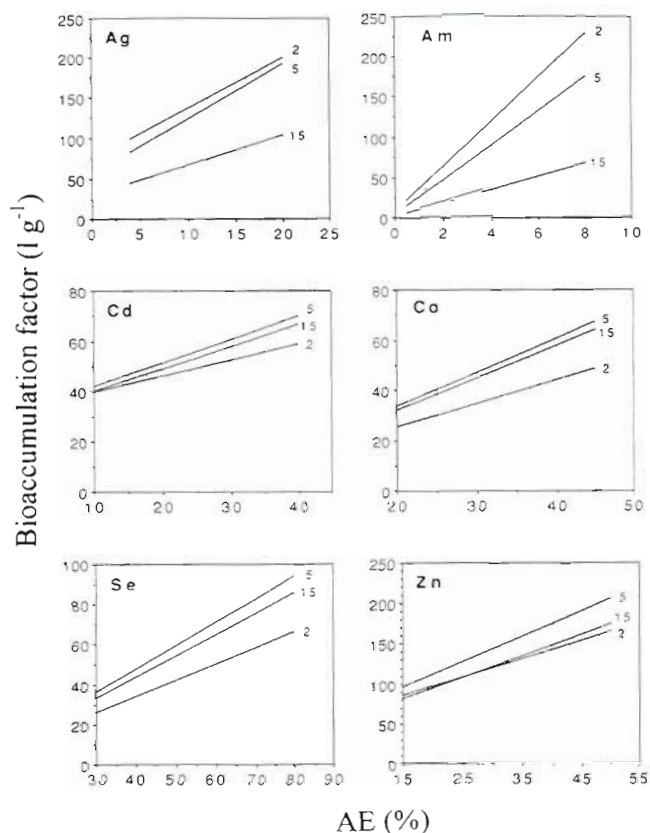


Fig. 13. *Mytilus edulis*. Relationship of the predicted bioaccumulation factor (BAF,  $\text{l g}^{-1}$  dry wt) of 6 trace elements in mussel soft parts and their AEs from ingested particles, for TSS loads of 2, 5, and 15  $\text{mg dry wt l}^{-1}$ . BAF calculated using Eq. (15)

change in BAF). Among the 6 elements, Cd is the least affected by AE (slopes of 0.2 to 0.3). Ag and Am are strongly affected at high TSS loads (slopes of 0.34 for Ag, 0.71 for Am) and less affected at lower TSS loads (slopes of 0.26 for Ag, 0.62 for Am) because of the relationship described above. For the other elements, the slopes are about 0.4 to 0.5 for Zn, and 0.7 to 0.8 for Co, respectively.

#### Salinity

Salinity can affect both metal influx rate from the dissolved phase and  $K_d$  for suspended particles. Kuwabara et al. (1989) found that the  $K_d$ s of Cd and Zn in South SFB increased by 1.3 and 1.6 $\times$ , respectively, when the salinity decreased from 32 to 20‰. However, variable effects of salinity have been reported for Ag (Smith & Flegal 1993), Se (Cutter 1989), Cd and Zn (Sung 1995, Wood et al. 1995). We assume that salinity will only affect metal influx rates from the dissolved phase and then calculated the change in BAF for salinities of 20 to 32‰ (typical of South SFB). These calculations

use the median  $K_d$  and AE for each metal (Fig. 10). By lowering the salinity from 32 to 20‰, the estimated BAFs increase by 35% for Ag, 14% for Am, 38% for Cd, 13% for Co, 1% for Se, and 24% for Zn. The relative contribution of dissolved metal uptake will increase from 50 to 63% for Ag, from 9 to 20% for Am, from 64 to 74% for Cd, from 14 to 24% for Co, from 2 to 3% for Se, and from 34 to 47% for Zn. These results suggest that the influence of salinity on metal bioaccumulation in mussels is small compared to other factors.

#### Partition coefficient

Variations in  $K_d$  reflect changes in metal partitioning in the dissolved and particulate phases (and thus metal concentrations in both phases; see Eq. 13). Therefore, metal influx rates from both the dissolved and particulate phases vary with  $K_d$ . We calculated the BAFs for each metal by increasing the  $K_d$  10 $\times$  (using the lowest  $K_d$  for each metal shown in Fig. 10), assuming a 5  $\text{mg l}^{-1}$  TSS load and an intermediate AE for each metal (see Fig. 10). The results indicate that this 10-fold increase in  $K_d$  would lead to BAF increases of 16% for Ag, 22% for Am, 145% for Cd, 575% for Co, 694% for Se, and 280% for Zn. The smallest effect of  $K_d$  on BAF was for the most particle-reactive elements (Ag and Am) because  $C_w$  decreases most for these elements with increasing  $K_d$  values (see Fig. 11). The greatest effect of increasing the  $K_d$  was for Co and Se. For both elements, which have relatively low  $K_d$  values, the  $C_w$  does not change appreciably as  $K_d$  increases, and >70% of the Co and >96% of Se is calculated to come from the food (Fig. 10), leading to sharp increases as particulate concentrations increase.

## DISCUSSION

#### Trace element assimilation from food

The AEs of the trace elements from natural seston were generally in the lower range of AEs measured for diverse phytoplankton foods, consistent with our previous observation that trace element assimilation is related to the nutritional quality of ingested food particles (Wang & Fisher 1996a). For example, AEs for algal food range between 4 and 34% for Ag, 0.7 and 6% for Am, 11 and 34% for Cd, 20 and 43% for Co, 15 and 72% for Se, and 16 and 48% for Zn (Wang & Fisher 1996a). Because natural seston consist of diverse biological and inorganic particles (including algal cells and resuspended sediment), it is unlikely that a single AE for each trace element can be applicable to all natural systems.



The cytoplasmic distribution directly influenced their assimilation of at least some elements from the natural seston, as observed elsewhere (Reinfelder & Fisher 1991, 1994). The 1:1 relationship between Se cytoplasmic distribution and its assimilation in mussels was consistent with a previous study showing a 1:1 relationship between the cytological distribution of Se in the diatom *Thalassiosira pseudonana* and its AE in mussels (Wang & Fisher 1996b), even though cytoplasmic Se and AEs from seston were lower than those measured from unialgal diets (Wang & Fisher 1996a). A possible explanation for the low cytoplasmic Se concentration is the shorter labeling period (36 h, under dark conditions), which may limit Se penetration into the algal cytoplasm. This element is mainly associated with soluble amino acids and small polypeptides (Wrench 1978, Fisher & Reinfelder 1991) in algal cytoplasm. The greatest deviation from the 1:1 relationship of AE and cytoplasmic distribution was found for Ag, Cd, and Zn, consistent with Wang & Fisher's (1996a) findings for these metals.

#### Trace element uptake from the dissolved phase

The present study employed a kinetic approach to determine trace element uptake rate from the dissolved phase. The short term exposure provided a best estimate of gross influx rates, without a pronounced influence from efflux. These influx rates are then suitable for use in a kinetic model. Most previous trace element uptake studies from the dissolved phase exposed mussels from days to weeks in the absence of food particles. In addition to being unsuitable to determine model coefficients (gross influx), biases can occur in long term exposures due to decreased filtration activity (starvation stress) or complexation with mussel metabolites/exudates or mucus. Conducting such experiments to directly determine  $C_{ss}$  is also problematic. Time to reach steady state can vary with the trace element and environmental conditions. For example, Regoli & Orlando (1994) showed that an equilibrium can be achieved in mussels following 2 wk transplantation to contaminated waters, but Tessier et al. (1993) observed that Cd equilibrium in the freshwater bivalve *Anodonta grandis* was not reached after 1 yr.

The influx rate from the dissolved phase increased linearly with dissolved concentration, as observed in juvenile mussels exposed for longer periods to dissolved Cd over a concentration range of 0.02 to 50  $\mu\text{g l}^{-1}$  (Fischer 1988) and in adult mussels exposed to Pu and Am (Bjerregaard et al. 1985). However, the kinetic approach provides the first quantitative measurement of dissolved uptake rate constants ( $k_u$ ) for trace elements in mussels. The uptake rate constant is a func-

tion of a mussel's filtering activity and absorption efficiency from the dissolved phase. Within a species, values of  $k_u$  represent the relative assimilability of trace elements from the dissolved phase. Because they are physiologically mechanistic, the constants can be objectively compared among species (if differences in filtration rates are known), elements, or environmental conditions. In mussels, the  $k_u$  values differed widely among trace elements, with  $\text{Ag} > \text{Zn} > \text{Am} \approx \text{Cd} > \text{Co} > \text{Se}$ . This is consistent with observations in the polychaete *Nereis diversicolor* in which uptake rates decrease in the order of  $\text{Hg} > \text{Cu} > \text{Ag} > \text{Cr} = \text{Pb} > \text{Zn} > \text{Cd} > \text{Ni} > \text{Co}$ , Se (Bryan 1984). Ag, Zn and Cd can bind strongly with sulfur groups in protein and show the greatest propensity for transport across membranes. Strong binding of these metals with intracellular pools such as metallothioneins may also increase their removal from ambient aqueous environments. In addition, the high  $k_u$  of Ag may be explained by its speciation in marine waters, which is dominated by a  $\text{AgCl}^0$  neutral charge complex (Turner et al. 1981, Byrne et al. 1988). This complex is very bioavailable to marine organisms due to its low polarity (Engel et al. 1981).

It is also notable that the absorption efficiencies from the dissolved phase estimated here are significantly lower than the assimilation efficiencies from food. However, given their high filtration rates (52 to 196  $\text{l g}^{-1} \text{d}^{-1}$ ; Widdows et al. 1995), mussels process large volumes of water, and so uptake from the dissolved phase may contribute significantly to metal accumulation in mussels. We are aware of no direct measurements of metal absorption efficiencies from the dissolved phase in marine bivalves.

The increase observed in metal influx rates at lower salinities is consistent with many previous reports (Phillips 1976, Jackim et al. 1977, Fischer 1986, 1988, Bjerregaard & Depledge 1994), however salinity had a smaller effect than other factors studied here. Other reports have suggested that changes in metal speciation are responsible for greater uptake rates at lower salinities (Wright 1995). Many studies have demonstrated that the free metal ion is the most bioavailable form in aquatic organisms, including marine mussels (Bjerregaard & Depledge 1994, Campbell 1995). In the present study, the observed increase in element uptake with decreasing salinity was similar for all trace elements that change speciation at low salinity (Ag, Cd, and Zn) and for elements that do not (Turner et al. 1981, Byrne et al. 1988). Se may be considered as a 'control' to monitor the influence of free ion on metal uptake at different salinities. Se was added in selenite form, which does not speciate differently at different salinities. Influx rates of Se, as well as those of Ag, Cd, Co and Zn, increased 1.6 to 1.9 times when the salinity decreased from 34 to 20‰. When the salinity was fur-

ther lowered to 15‰, Ag and Cd exhibited influx rate decreases similar to Se. In fact, salinity change in the 15 to 35‰ range has only a small influence on free ion concentrations of trace metals, so trace element speciation may not be the best explanation for the observed decrease in metal influx at high salinity. Wright & Zamuda (1987) also showed that the salinity effect can be independent of Cu free ion activity in seawater. They maintained a relatively constant Cu free ion activity at different salinities, and measured its accumulation in oysters *Crassostrea virginica* and clams *Mya arenaria*. Both species accumulated significantly more Cu at lower salinity (but at constant ionic activity), indicating that the salinity effect is independent of Cu free ion activity in seawater.

A possible mechanism underlying the increased influx at low salinity is the decreasing osmolarity and increasing cell membrane permeability at lower salinities (Chan et al. 1992). George et al. (1978) demonstrated that a decrease in osmolarity was responsible for an increase in Cd uptake in mussels at low salinities. When the osmolarity was held constant, Cd uptake was independent of salinity over a range of 15 to 35‰.

No significant difference in influx rate was detected at different concentrations of DOM and for different batches of seawater. The UV irradiation only removed about 50% of the DOM from the seawater, and this may not have been sufficient to result in a significant change in free ion activity. However, Zamuda et al. (1985) showed that over a 24 h exposure to 10  $\mu\text{g Cu l}^{-1}$ , the Cu accumulation rate in oysters *Crassostrea virginica* doubled when the seawater was UV irradiated (removing 50% of the DOM). Absil et al. (1993) also found that metal complexation by natural ligands was important in reducing Cu bioavailability to *Macoma balthica* exposed to dissolved Cu for 28 d, with Cu uptake rates decreasing by >50% when DOM levels increased by >5-fold.

### Trace element efflux

Depuration patterns for whole mussels (soft parts and shell) of the trace elements were characterized by 1 to 3 compartments, consistent with many previous observations in marine bivalves (Young & Folsom 1967, Dahlgaard 1981, 1986, Bjerregaard et al. 1985). The first compartment was probably dominated by egestion of unassimilated materials and rapid desorption of surface bound material. The depuration of Cd after 6 or 7 d of uptake from food or the dissolved phase, however, indicated that this element was evident in only 1 compartment, perhaps reflecting its sequestration principally by metallothionein.

Fowler et al. (1975) demonstrated that the excretion kinetics of  $^{65}\text{Zn}$  in the shrimp *Lysemata seticauda* was dependent on the route of Zn exposure, due to different equilibration times of  $^{65}\text{Zn}$  with stable Zn pools in the animals. Neither exposure time (12 h vs 6 d) nor route of uptake (dissolved vs food) had a significant effect on the efflux rate in mussels in our study, suggesting that radiotracers were able to partition rapidly into the slowest compartment. The proportion of radioisotope in the slowest compartment, however, increased with duration of exposure, as observed in many other studies (Cutshall 1974, Amiard 1978, Nugegoda & Rainbow 1989).

The efflux rate constant of Ag was greater after particulate ingestion than after uptake from the dissolved phase, in contrast to the bivalve *Scrobicularia plana*, in which Amiard (1978) found that  $^{110m}\text{Ag}$  was lost faster following uptake from the dissolved phase. The difference in Ag efflux may be due to its binding with different ligands, as shown in oysters (Berthet et al. 1992). Specifically, Ag from food may bind with metal-rich granules ( $\text{Ag}_2\text{S}$ ) in the cell lining of the digestive tract, which then release Ag into the gut lumen for egestion by defecation. Ag accumulated from the dissolved phase may be stored in the kidneys in insoluble form (George et al. 1986), and then excreted at a rate slower than Ag defecation. Our unpublished data also suggest that excretion was the major pathway of Ag physiological loss after dissolved uptake, whereas defecation was dominant after food uptake.

The biological half-lives of the 6 trace elements in the mussels measured in this study were within the range of values measured in previous studies (Table 9). It is difficult to directly compare efflux rates among different studies because different investigators often employ different experimental conditions, some or all of which may strongly influence efflux rates. Recently, Fisher et al. (1996) showed that efflux rates of 7 trace elements from the mussel *Mytilus galloprovincialis* in the laboratory and in the field are directly comparable, particularly for mussel soft parts.

### Modeling trace element bioaccumulation in mussels

The kinetic modeling approach allows an assessment of the effects of changes in geochemical and biological factors on the bioaccumulation of various metals. Our kinetic model predicted metal concentrations in marine mussels that are comparable to concentrations measured in various monitoring programs, suggesting that metal accumulation in mussels can be accurately predicted using the physiological and geochemical parameters identified in the kinetic model. It is clear that 2 physiological parameters, metal absorp-

Table 9. Comparison of biological half-lives ( $tb_{1/2}$ ) of trace elements in whole mussels (shell and soft parts) from various studies. *M.e.*: *Mytilus edulis*; *M.c.*: *Mytilus californianus*; *M.g.*: *Mytilus galloprovincialis*

Element	$tb_{1/2}$ (d)	Species	Condition	Source
Ag	22–38	<i>M.e.</i>	Laboratory depuration	This study
Ag	45–58	<i>M.e.</i>	Field depuration	Dahlgaard (1986)
Ag	17	<i>M.g.</i>	Field depuration	Whitehead et al. (1988)
Ag	24–27	<i>M.g.</i>	Field depuration	Fisher et al. (1996)
Ag	4–25	<i>M.g.</i>	Laboratory depuration	Fisher et al. (1996)
Am	35–39	<i>M.e.</i>	Laboratory depuration	This study
Am	88	<i>M.e.</i>	Laboratory depuration	Bjerregaard et al. (1985)
Am	300	<i>M.e.</i>	Field depuration	Clifton et al. (1983)
Am	480	<i>M.g.</i>	Field depuration	Guay & Fowler (1981)
Am	141–195	<i>M.e.</i>	Field depuration	Dahlgaard (1986)
Am	38–64	<i>M.g.</i>	Field depuration	Fisher et al. (1996)
Am	15–61	<i>M.g.</i>	Laboratory depuration	Fisher et al. (1996)
Cd	67–78	<i>M.e.</i>	Laboratory depuration	This study
Cd	1155	<i>M.g.</i>	Laboratory depuration	Fowler & Benayoun (1974)
Cd	347	<i>M.g.</i>	Field depuration	Fowler & Benayoun (1974)
Cd	104	<i>M.e.</i>	Food ingestion	Borchardt (1983)
Cd	122–190	<i>M.e.</i>	Dissolved uptake	Borchardt (1983)
Cd	46–822	<i>M.g.</i>	Field depuration	Fisher et al. (1996)
Cd	16–60	<i>M.g.</i>	Laboratory depuration	Fisher et al. (1996)
Co	45–72	<i>M.e.</i>	Laboratory depuration	This study
Co	580	<i>M.e.</i>	Field depuration	Dahlgaard (1981)
Co	57–72	<i>M.e.</i>	Laboratory depuration	Van Weers (1973)
Co	79–88	<i>M.e.</i>	Field depuration	Dahlgaard (1986)
Co	55–65	<i>M.g.</i>	Field depuration	Fisher et al. (1996)
Co	12–35	<i>M.g.</i>	Laboratory depuration	Fisher et al. (1996)
Se	28–39	<i>M.e.</i>	Laboratory depuration	This study
Se	81	<i>M.g.</i>	Field depuration	Fowler & Benayoun (1976)
Zn	44–66	<i>M.e.</i>	Laboratory depuration	This study
Zn	82	<i>M.c.</i>	Field transplantation	Young & Folsom (1967)
Zn	87	<i>M.e.</i>	Field depuration	Dahlgaard (1986)
Zn	48–60	<i>M.e.</i>	Laboratory depuration	Van Weers (1973)
Zn	67	<i>M.e.</i>	Field depuration	Dahlgaard (1986)
Zn	41–52	<i>M.g.</i>	Field depuration	Fisher et al. (1996)
Zn	11–1291	<i>M.g.</i>	Laboratory depuration	Fisher et al. (1996)

tion efficiency from the dissolved phase and assimilation efficiency from the particulate phase, are critical criteria in assessing metal bioavailability to aquatic animals.

### Cadmium

Under the different conditions that can occur in SFB, the kinetic model predicted that Cd concentrations in mussels would vary from 2.7 to 10.1  $\mu\text{g g}^{-1}$ , a range remarkably similar to that observed in the NS&T monitoring program. A change in food sources that caused AE to shift from 0.1 to 0.3 would cause a 1.5 $\times$  increase in tissue concentrations (2.7 vs 4.5  $\mu\text{g g}^{-1}$ ). If the highest dissolved concentrations occurred during reduced salinity when a food source was present with highly bioavailable Cd, the maximum tissue burden expected in mussels in the Bay would be about 10.1  $\mu\text{g g}^{-1}$ .

In the above scenario for SFB, uptake of dissolved Cd was calculated to contribute >50 to 80% of the total Cd

burden in mussel tissues. Estimates of the relative importance of Cd uptake from food and solution have varied in previous studies. Borchardt (1983) used  $^{109}\text{Cd}$  and  $^{115}\text{Cd}$  to radiolabel algal cells and water, respectively, and then measured the relative accumulation of these 2 isotopes in mussel tissues. The Cd concentration he derived from food uptake was <1% of the total Cd accumulated. Other studies also concluded that uptake of particulate Cd contributed <10% of the total Cd body burden in mussels (Janssen & Scholz 1979, Riisgård et al. 1987). These measurements examined only 1 food species. Thomann et al. (1995), using a steady-state model of mussel accumulation of metals, concluded that >90% of Cd is obtained from ingested food particles; they used only 1 AE value for each metal, 1 IR value and, lacking direct data on uptake rates from the dissolved phase, used bioconcentration factors to estimate influx from the dissolved phase.

Cossa (1988) summarized a linear relationship between Cd concentration in mussel tissue and Cd concentration in seawater from various coastal loca-



tions, which can be described by  $C (\mu\text{g g}^{-1}) = 0.074 \times C_w (\text{ng l}^{-1}) + 0.390$ , where  $C_w$  is the Cd concentration in seawater and  $C$  is the Cd concentration in mussel tissues. Of course, such a correlation does not necessarily mean that all Cd uptake was from seawater. The kinetic model projected concentrations very similar to the calculation from his empirical relationship, especially using a  $K_d$  value of  $5 \times 10^3$  and an AE of 40% or a  $K_d$  value of  $10^4$  and an AE of 20%. The model predicted that 45% of Cd in mussels was obtained from ingestion of food and 55% via uptake from water in these situations.

### Selenium

Modeling Se accumulation based only on selenite uptake adequately explained the Se concentration in field-collected mussels because virtually all of the Se should be accumulated from ingested food (>96%). Luoma et al. (1992) showed that Se concentrations in the clam *Macoma balthica* in SFB could be accounted for primarily by assimilation of Se from ingested food, consistent with other observations of Se accumulation in aquatic invertebrates (Fowler & Benayoun 1976, Zhang et al. 1990). The dominance of Se uptake from food is mainly due to the slow uptake rate from the dissolved phase and the high assimilation efficiency. The calculated dissolved uptake constant for Se was 4 to 50× lower than the other 5 elements. The ultimate source of Se for mussels is probably selenite, but first it must be accumulated out of the dissolved phase by phytoplankton (Fisher & Reinfelder 1991).

### Zinc

At low dissolved Zn concentrations ( $0.5 \mu\text{g l}^{-1}$ ), the projected Zn concentration in mussel tissues at Dumbarton Bridge station is within the range of values measured in monitoring programs, whereas at high Zn concentrations ( $1.7 \mu\text{g l}^{-1}$ ; Flegal et al. 1991), the projected mussel concentration exceeds measured concentrations, especially when using high estimates of AE (Table 8). It is noteworthy, however, that higher Zn concentrations ( $120$  to  $340 \mu\text{g g}^{-1}$ ) in mussels were measured at the Emeryville Station in SFB (T. P. O'Connor pers. comm.). However, the predicted Zn concentrations ( $34$  to  $157 \mu\text{g g}^{-1}$ ) in LIS are fully within the range of actual concentrations ( $52$  to  $142 \mu\text{g g}^{-1}$ ) measured by the Mussel Watch Program (Goldberg et al. 1983).

The relative contribution of Zn predicted from food uptake was about 50 to 72% under different environmental scenarios. Thomann et al. (1995), using the

same approach as for Cd uptake (see above), estimated that >90% of Zn in mussels is accumulated from food. Our model indicates that  $K_d$  is a critical parameter affecting the relative importance of each uptake pathway for Zn. The relative uptake from the dissolved phase decreases by 2.8× when the  $K_d$  increases by 5×. In this model, we employed a moderate range of  $K_d$  for Zn ( $1$  to  $5 \times 10^4$ ; Balls 1989, Kuwabara et al. 1989, Benoit et al. 1994), comparable to its concentration factor in phytoplankton (Fisher & Reinfelder 1995), but lower than recent unpublished measurements in SFB (B.-G. Lee, A. van Geen & S. Luoma unpubl.).

### Silver

A varying contribution from the dissolved phase was noted for Ag (30 to 70%) in our study. Because this metal is very particle-reactive and exhibits variable partitioning in natural seston,  $K_d$  plays the most significant role in the relative importance of uptake of dissolved Ag. Other experimental studies suggested that Ag was taken up by grass shrimp *Palaemonetes pugio* and oysters *Crassostrea virginica* and *C. gigas* mainly from the dissolved phase (Martoja et al. 1988, Abbe & Sanders 1990, Connell et al. 1991). Abbe & Sanders (1990) showed that oysters did not accumulate Ag from sediments or from algal food during a 14 to 21 d uptake period. In their study, desorption of Ag from ingested particles was not important in removing Ag from ingested particles, thus most Ag ingested by the oysters was subsequently egested as feces. The variable results of such studies suggest that environmental conditions may determine which pathway(s) of Ag dominate uptake, even more so than for other elements.

### Americium and cobalt

There have been a few measurements of Am and Co concentrations in seawater or in mussels, but not both, in the same time and place (Goldberg et al. 1983, Flegal et al. 1991, Valette-Silver & Lauenstein 1995), so we cannot at present use our model to predict concentrations of these metals in the field. Although the influx rate of Am from the dissolved phase was high and its AE from food was low, its very high  $K_d$  results in a low dissolved concentration, and therefore the particulate phase is more important than the dissolved phase (4:1) in Am accumulation in mussels. For Co, the dominance of the particulate pathway may be attributed to its low dissolved influx rate and high AE.

In summary, this study demonstrates that the kinetic model can be used to describe trace element bioaccu-



mulation in mussels. Rate constants (e.g. for influx and efflux) determined in the laboratory are valuable for understanding and predicting metal accumulation in marine bivalves. Although the data required by this model are relatively extensive, the model is robust in that it can incorporate various environmental conditions that may affect metal bioaccumulation, such as temperature, salinity, dissolved organic matter, and seston load. Furthermore, it avoids problems typically associated with an equilibrium approach in describing metal accumulation in aquatic animals. Comparison of influx rate constants for various uptake pathways enables a direct estimation of the relative importance of each uptake pathway. Our calculations illustrate that trace elements vary widely in their accumulation patterns in mussels and that both dissolved and particulate metals are appreciably accumulated in mussels. Water quality criteria based solely on dissolved metal concentrations are therefore inadequate to assess metal bioavailability to marine bivalves. The relative importance of each source is principally related to metal partitioning in food particles, metal assimilation efficiency and to a lesser degree, the seston load. Other factors may also affect the contribution from each source by influencing the influx rate (e.g. the effects of salinity on the influx rate from the dissolved phase). The model demonstrates that the total concentration of trace element in the water column ( $C_i$ ), the assimilation efficiency in the mussels (AE), and the seston load (TSS) are all major parameters determining metal accumulation in mussels. Thus, with this model it is possible to further elucidate rates and routes of metal accumulation in mussels, and this information can be used to interpret the voluminous data being generated by national monitoring programs.

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