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COMPARISON OF SELENIUM BIOACCUMULATION IN THE CLAMS *CORBICULA FLUMINEA* AND *POTAMOCORBULA AMURENSIS*: A BIOENERGETIC MODELING APPROACH

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Abstract—Selenium uptake from food (assimilation efficiency) and dissolved phase (influx rate) as well as loss kinetics (efflux rate) were compared between two bivalves, *Corbicula fluminea* and *Potamocorbula amurensis*. The effects of salinity and temperature on these kinetic parameters for both clam species also were evaluated. The Asiatic clam, *C. fluminea*, more efficiently assimilated Se associated with algae (66–87%) than Se associated with oxic sediments (20–37%). However, no consistent difference was found between Se assimilation efficiencies from both food types (19–60%) for *P. amurensis*. The temperature and salinity had a minor influence on the Se assimilation from ingested food. However, the effects of temperature and salinity were more evident in the uptake from dissolved sources. The influx rate of Se(IV) increased by threefold with the increase of temperature from 5 to 21°C for *C. fluminea*. The increase of salinity from 4 to 20 psu decreased the uptake rate constant (k_u) of Se in *P. amurensis* from 0.011 to 0.005 L/g/h, whereas salinity change (0–8 psu) had a negligible effect on the Se influx rate of *C. fluminea*. The Se influx rate of *P. amurensis* decreased by half with the 3.5-fold increase in tissue dry weight. The rate constant of loss was greater for *P. amurensis* (0.029/d at 8 psu) than for *C. fluminea* (0.014/d at 0 psu and 0.01/d at 8 psu). A bioenergetic model suggests that dietary uptake is the dominant pathway for Se bioaccumulation in the two clams in San Francisco Bay and that interspecies differences in Se bioaccumulation can be explained by differences in food ingestion rates.

Keywords—Selenium Bioaccumulation Bioenergetic model *Corbicula fluminea* *Potamocorbula amurensis*

INTRODUCTION

Selenium is an essential element for most organisms, but at relatively low environmental concentrations, it may cause severe reproductive toxicity, including impaired egg hatchability and embryo deformities, especially to upper-trophic-level organisms [1–3]. The difference between essential and potentially hazardous Se concentrations is relatively narrow [4]. The chemical speciation of Se is particularly important, because the biotic and abiotic reactivity of Se are functions of its chemical form [5]. Selenium exhibits complex biogeochemical cycling, with several oxidation states in aquatic environments [selenate (Se^{6+}), selenite (Se^{4+}), elemental Se (Se^0), and reduced organoselenide (Se^{2-})]. Bioaccumulation of Se occurs primarily from the base of food chains, where microorganisms transform dissolved selenite and selenate (to a lesser extent) into organoselenide within the cells. Organoselenide is then transferred via the food chain and is often biomagnified to upper-trophic-level invertebrates [4,6,7]. Therefore, understanding the physicochemical and biological factors influencing the transfer of Se from the base of the food chain to herbivorous organisms is essential for the assessment of Se bioaccumulation and its potential effects on upper-trophic-level organisms [3].

Selenium has been introduced to North San Francisco Bay (SFB; USA) from agricultural drainage systems as well as from the effluent of oil refineries [5]. Various bivalve species have been employed to monitor Se bioavailability in North SFB and have displayed a wide range of spatial and temporal variations in tissue Se concentrations. For example, tissue Se concentrations in the Asian clam, *Potamocorbula amurensis*, were the highest among the bivalves in the North SFB and varied both spatially (6–20 μg/g) and seasonally (5–20 μg/g) [8]. The greatest Se concentrations in the clams occurred during low-water-flow years near the Carquinez Strait; tissue concentrations in *P. amurensis* often exceeded dietary thresholds (~10 μg/g) for adverse reproductive effects in predators, such as fish and waterfowl, because of prey consumption [1]. For this reason, *P. amurensis*, the dominant bivalve in SFB, poses a potential threat to upper-trophic-level organisms, including sturgeon, Dungeness crab, and diving ducks [8]. However, it is not understood why *P. amurensis* bioaccumulate Se far more than other bivalves, such as *Corbicula fluminea*, which coexist with *P. amurensis* at the same site [5].

A bioenergetic-based kinetic model has been developed to delineate the relative importance of uptake pathways and to quantify the effects of biological and environmental variables on the metal bioaccumulation in various indicator species [9–11]. For example, with this approach, Luoma et al. [9] demonstrated that 98% of Se in the benthic clam, *Macoma balthica*, could be explained by accumulation via ingestion of contaminated particles with minor contributions from dissolved sources. Furthermore, this modeling approach can be applied to explain different levels of tissue metal concentrations in different species from the same environment. To compare metal bioaccumulation among different animal species, estimation of some fundamental physiological parameters specific for each species is required.

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Enriched Se in bivalves often occurs in the upper reach of North SFB near Carquinez Straight, where physicochemical conditions, such as salinity and temperature, vary dynamically over time and space. Therefore, to assess the risk of Se contamination in SFB, a quantitative understanding must be developed regarding how external environmental factors and internal biological factors, such as body size and assimilation process, affect Se bioaccumulation in the bivalves, an important food source for aquatic dependent wildlife [9,12].

The present study compared biokinetic parameters for Se uptake from food (assimilation efficiency [AE]) and dissolved phase (influx rate) as well as loss (efflux rate) between two bivalves, *P. amurensis* and *C. fluminea*. Additionally, AEs from ingested particles were compared between Se associated with fresh algae and sediments. The influences of salinity and temperature on these kinetic parameters also were evaluated. The acquired biokinetic parameters were incorporated into a dynamic, multipathway bioaccumulation model to predict tissue Se levels between the two clam species and to compare those predictions with the levels observed in SFB. These two invasive species coexist in SFB, process a large volume of water and particles, concentrate Se at high concentrations, and serve as important food sources for benthivorous predators. These two clams have been employed in numerous biomonitoring and ecotoxicological studies [3,8,13,14].

MATERIALS AND METHODS

Experimental animals

The Asiatic clam, *C. fluminea* (shell size, 18–22 mm), was collected from a local freshwater stream located in San Jose (CA, USA), and the estuarine clam, *P. amurensis* (12–20 mm), was collected from a site in North SFB (U.S. Geological Survey Station 8) [13], approximately one week before each experiment. On returning to the laboratory, the clams were gradually acclimated to the experimental salinity (0, 4, or 8 psu for *C. fluminea* and 4, 8, or 20 psu for *P. amurensis*) and temperature (5, 13, or 21°C for both species). The clams were fed diatoms (*Phaeodactylum tricornutum*) during the acclimation period. The artificial freshwater media (0 psu) were prepared using the Fraquil recipe [14], and the saline media (4, 8, and 20 psu) were made by diluting (pore size, 0.45 μm) natural seawater (35 psu; collected from Long Marine Laboratory, University of California, Santa Cruz, CA, USA) with the freshwater media in appropriate ratios. The experimental salinity was chosen for each clam's optimal salinity range.

Radiolabeled food

Radiolabeled algae was prepared by adding 37 kBq of radioactive ^{75}Se ($Na_2{\cdot}^{75}SeO_3$) to a 1-L flask containing seed culture of the diatom *P. tricornutum* and was maintained at 20°C for 7 d. The culture media was 12-psu seawater enriched with f/2 nutrients (N, P, Si, vitamins, and trace metals) modified from that described by Guillard [15]. The spiked radioactive Se concentration (0.04 nM) was two orders of magnitude lower than that observed in SFB waters. The surface oxidized sediments (<63 μm), collected from the same stream where *C. fluminea* were sampled, were resuspended and equilibrated with 37 kBq of ^{75}Se in 1 L of media at 0, 4, 8, or 20 psu. These media were shaken periodically and maintained at 20°C in the dark for 10 d.

The radiolabeled algae or sediments were filtered on polycarbonate membrane filters (pore size, 0.45 μm) and rinsed three times with unlabeled media. The filtered particles were then resuspended in the unlabeled media for feeding experiments with the appropriate salinity and temperature. Some of the radiolabeled media with their labeled algae were saved for later radiolabeling of the clams to measure the efflux rate.

Uptake from food

The AE of clams for Se from ingested algae or sediments was estimated using a previously established pulse-chase procedure [9,16]. The effect of salinity on Se AE for *C. fluminea* (0, 4, or 8 psu) or *P. amurensis* (4, 8, or 20 psu) was always evaluated at 13°C. The effect of temperature on Se AE (5, 13, and 21°C) for *C. fluminea* and *P. amurensis* was evaluated in freshwater media and in 20-psu seawater, respectively. Additionally, *C. fluminea* were fed two types of food mixtures (radiolabeled algae mixed with unlabeled sediment or radiolabeled sediment mixed with unlabeled algae) in freshwater media at 13°C. These treatments were included to determine whether the presence of different food types would influence the AE for Se of each food.

Thirty *P. amurensis* (13.3 ± 0.5 mm; mean ± standard deviation) or *C. fluminea* (18.4 ± 0.5 mm) were introduced into each of the feeding chambers containing resuspended radioactive food in appropriate media. The clams were allowed to ingest radioactive food for approximately 2 h. Following the radioactive feeding, the clams were randomly divided into eight groups, each composed of three clams, and the radioactivity of each group was measured. Four groups of clams were dissected immediately to determine the ratio of radioactivity between shells and soft tissue, and the remaining four groups of clams were depurated for 48 h in appropriate unlabeled media with unlabeled food. Clams in the depuration chambers were separated from feces by the meshed screen to minimize the ingestion of feces. During the 48-h depuration periods, radioactivity remaining in the clams and egested radioactivity in feces were periodically determined. The depurated clams were dissected for the determination of radioactivity in tissues and shells.

During the feeding of resuspended radiolabeled food, radioactive Se could be released from the food particles into the media. To estimate Se uptake from this source, additional groups of clams were exposed for 2 h to the filtered (pore size, 0.45 μm) feeding media that had been used previously for pulse-chase feeding experiments. The contribution of dissolved Se released from food particles generally was negligible (<2%) compared to the total assimilated Se from food.

Uptake from dissolved phase

To determine the influence of selenite concentration on influx rate, both clam species were exposed to a range of nominal Se concentrations (0.02, 0.1, 0.5, 1.5, and 10 μg/L for *C. fluminea* and 0.09, 0.13, 0.43, 1.1, 3.5, and 10.3 μg/L for *P. amurensis*). Experimental medium was prepared by adding appropriate amounts of stable selenite solution (1,000 mg/L) to 2 L of filtered (pore size, 0.45 μm) water. Radioactive ^{75}Se was added as a tracer of stable selenite; each container received 74 kBq of ^{75}Se as $Na_2{\cdot}^{75}SeO_3$ dissolved in weak acid. The pH of the experimental media was adjusted to 8.0 by the addition of adequate amounts of 0.1 N NaOH solution.

The effect of salinity on the Se influx rate for *C. fluminea* (0, 4, or 8 psu) or *P. amurensis* (4, 8, or 20 psu) was evaluated at 13°C. The effect of temperature (5, 13, and 21°C) was evaluated only for *C. fluminea* in freshwater media. A reduced

number of Se exposure concentrations was used for some treatments because of the logistical limitations of the radioactive experiment. Five replicates of three similar-sized *C. fluminea* (19.1 ± 1.2 mm) and *P. amurensis* (16.6 ± 1.5 mm) were used for each experimental treatment. Additionally, the effect of clam size (13.1 ± 0.4, 15.3 ± 0.9, 17.8 ± 0.6, and 19.6 ± 0.5 mm) on the Se influx rate was evaluated only for *P. amurensis* in 20-psu media at 13°C.

The clams were exposed to the Se-labeled media for 6 h under the respective experimental conditions. Radioactivity in experimental media was monitored periodically by sampling 2 ml of the exposure media; the radioactivity always changed by less than 10%. The clams were removed from the media after the exposure and rinsed with unlabeled water, and the radioactivity in the clam tissues and shells was determined.

Efflux of assimilated metal

To determine the physiological turnover rate (efflux rate) of Se assimilated in clam tissues, both clam species were exposed to radiolabeled food (algae and sediment) and media for 6 d and then depurated for 21 d. *Corbicula fluminea* (18.8 ± 0.4 mm) was radiolabeled and depurated in freshwater (0 psu) or seawater (8 psu) at 13°C, and *P. amurensis* (11.7 ± 0.4 mm) was depurated only in seawater (8 psu) at 13°C. Following the 6-d exposure, clams were removed from the aquarium and rinsed with filtered water. The clams were placed in a large depuration chamber containing 20 L of unlabeled media and maintained for 21 d. During the depuration periods, radioactivity of whole-body and tissue/shell partitioning was determined periodically. The temporal changes in the radioactivity of soft tissue were estimated by multiplying whole-clam radioactivity by the ratio of tissue to shell radioactivity obtained from clams [17].

Radioactivity measurement

Radioactivity was determined using a gamma counter equipped with a 3-inch, well-type, NaI crystal detector (1480 Wizard; Wallac, Turku, Finland). Photon emissions of ^{75}Se were determined at 244 keV. The counting times for all samples were 2 min. Propagated counting errors were less than 5%. All measured radioactivity was corrected for counting efficiency and decay and then expressed in terms of unit dry weight of soft tissue.

Data analysis

The AE was calculated using two independent methods [16]. The first value (AE_1) was calculated by dividing the final tissue radioactivities (A_f) remaining at $t = 48$ h by the initial ingested activities (A_0) estimated from the whole-body radioactivity at $t = 0$, corrected for the activity partitioned on the shell:

$$AE_1\ (\%) = (A_f - A_{diss})/(A_0 - A_{diss})\cdot 100 \quad (1)$$

where A_{diss} is the radioactivity in soft tissue of the clams exposed to filtered feeding media. The A_{diss} term was necessary to correct radioactivity obtained from dissolved Se released from radiolabeled food during feeding experiments. The second value (AE_2) was estimated by dividing the final tissue activities (A_f) by the sum of final tissue radioactivity (A_f) and radioactivity depurated as feces (A_{feces}) during the 48-h depuration in clean media:

$$AE_2\ (\%) = (A_f - A_{diss})/(A_{feces} + A_f - A_{diss})\cdot 100 \quad (2)$$

where the initial amount of ingested particles is assumed to be the sum of A_{feces} and A_f.

The Se influx rates from dissolved sources can be expressed as

$$I_w = k_u \cdot C_w^b \quad (3)$$

where I_w is the metal influx rate (μg/g/d), C_w is the Se concentration in water (μg/L), b (power coefficient) is the slope of the log–log relationship between I_w and C_w, and k_u (L/g/d) is considered to be the rate constant for uptake from the dissolved source if the slope (b) is close to one.

The physiological turnover rates of assimilated Se in clam tissue were described by a first-order exponential function:

$$R_t = R_0 \cdot e^{-k_e \cdot t} \quad (4)$$

where R_t is the fraction of Se retained in clam tissue at day t, R_0 is constant, k_e is the rate constant of loss, and t is time (d). Because the slope (k_e) is changed during the depuration, a multicompartment model can be applied.

Multipathway bioaccumulation model

A dynamic multipathway bioaccumulation model was used to evaluate the influence of geochemical and biological factors on Se bioaccumulation and to compare the tissue Se concentrations observed in the *C. fluminea* and *P. amurensis* from SFB. Tissue Se in the clams was evaluated using the first-order, multipathway bioaccumulation model [18]:

$$dC_a/dt = k_u \cdot C_w + (AE \cdot IR \cdot C_f) - (k_e + g)\cdot C_a \quad (5)$$

where C_a is the tissue Se concentration in the clams at time t, k_u is the rate constant of Se uptake from dissolved phase, C_w is Se concentration in dissolved phase, AE is Se AE, IR is food ingestion rate, C_f is Se concentration in food, k_e is the rate constant of loss, and g is the growth rate constant. At steady state, Equation 5 can be simplified to predict steady-state tissue Se concentrations ($C_{a,ss}$):

$$C_{a,ss} = \frac{k_u \cdot C_w + (AE \cdot IR \cdot C_f)}{k_e + g} \quad (6)$$

Unless otherwise noted, statistical significance was set at $\alpha = 0.05$. Dry weight–based concentrations were used for all tissue data. Statistica® (StatSoft, Tulsa, OK, USA) was used for all statistical analyses (Student's t test, one-way analysis of variance test and regression analysis).

RESULTS

Assimilation from dietary source

The difference between AE results calculated by different methods (AE_1 and AE_2) was always less than 20% for both clam species. The difference between the AE_1 and AE_2 data was not significant for *P. amurensis*. Some AE_2 data for *C. fluminea*, however, were significantly higher than AE_1 data ($p < 0.05$), although the differences were mostly less than 10%. The mean of AE_1 and AE_2 was used for comparison and model calculation in the present study (Fig. 1).

Corbicula fluminea assimilated Se more efficiently from algal food than from sediment particles, regardless of temperature and salinity (Fig. 1). Generally, salinity and temperature did not significantly influence the Se AE (%) in *C. fluminea*. The only exception to this was that the AE from algae at 8 psu was significantly lower than that at 0 or 4 psu. The AE of Se by *P. amurensis* was mostly within 52 to 61% regardless of food type, temperature, and salinity. The excep-

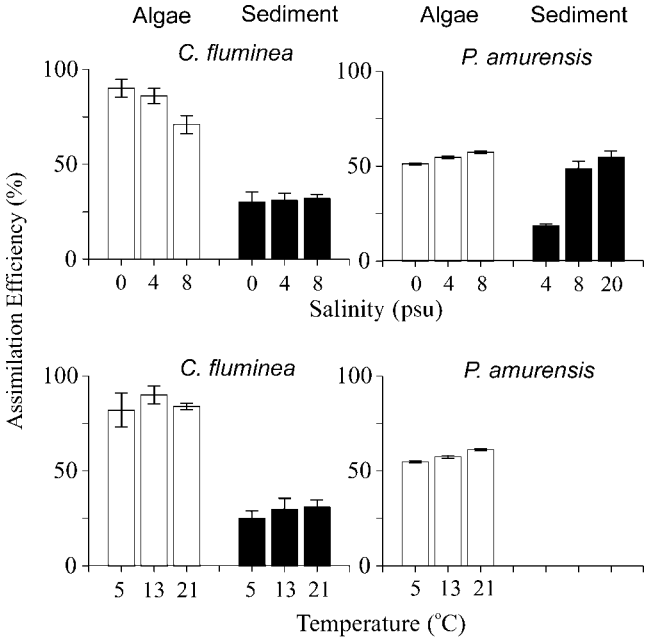

Fig. 1. Comparison of mean assimilation efficiencies of Se by *Corbicula fluminea* and *Potamocorbula amurensis* fed algae or oxic sediment at three different salinities (0, 4, and 8 psu) and a fixed temperature (13°C) or at three different temperatures (5, 13, and 21°C) at a fixed salinity (0 psu for *C. fluminea* and 20 psu for *P. amurensis*). Error bar indicates the standard deviation ($n = 4$).

tion was that *P. amurensis* assimilated Se significantly less from ingested sediments in media at 4 psu than the last of the treatments. From the algal food, *C. fluminea* assimilated Se with higher efficiency (70–90%) than did *P. amurensis* (51–57%), whereas the two clams did not show consistent differences in Se AEs from sediment particles (Fig. 1). The AEs of Se in food mixtures (radiolabeled algae mixed with unlabeled sediment or radiolabeled sediment mixed with unlabeled algae) for *C. fluminea* were not significantly different from Se AEs of the radiolabeled food type without an unlabeled counter food type (data not shown).

Uptake from dissolved phase

The influx rate of Se (IV) increased with the increase in the selenite concentrations in media. The slopes (b) in all the relationships between the log Se influx rate into clam tissues and the log selenite concentration in media (Eqn. 3) were close to one (Table 1). Therefore, we assumed that the selenite influx rates in both clams increased linearly with the exposure concentration and estimated the uptake rate constants (k_u) from Equation 3 (Table 1). The influx rate constant (k_u) of Se in *C. fluminea* increased by fourfold as temperature increased from 5 to 21°C (Table 1). However, the salinity change (0–8 psu) did not significantly influence the Se influx to clam tissues or partitioning to the shell in *C. fluminea* (data not shown). In contrast, the k_u in *P. amurensis* decreased by approximately half with an increase of salinity from 4 to 20 psu (Table 1). The amount of adsorbed Se on the shells of *P. amurensis* decreased by 30%, with the increase of salinity from 4 to 20 psu being consistent with tissue data (data not shown). The k_u values of Se in *P. amurensis* were 5.8- and 3.3-fold higher than that in *C. fluminea* at 4 and 8 psu, respectively (Table 1). Body size significantly influenced the Se influx in *P. amurensis*; the k_u for Se influx in *P. amurensis* decreased by half as the mean shell length increased from 13.1 to 19.6 mm (Table 1).

Efflux of assimilated Se

The clams defecated most of the unassimilated radioactive particles from digestive tracts within the 2-d depuration period in the assimilation experiment. Therefore, the radioactivity lost from the clams after the 2-d depuration was considered to be ''physiologically turned over'' Se. These initial loss data were excluded when calculating the k_e. The patterns of Se efflux over time could be described by a first-order exponential function with two-compartment loss models for both *C. fluminea* and *P. amurensis* (Fig. 2). The efflux was fast during the initial 7-d depuration and but slowed thereafter in both species. The rate constant of Se efflux (k_e) from the slowly exchanging compartment for *P. amurensis* in seawater at 8 psu and 13°C was 4.6-fold the k_e for respective *C. fluminea*. The Se efflux rate in *C. fluminea* decreased by half as the salinity increased from 0 to 8 psu (Fig. 2).

Biokinetic model results

A simple bioaccumulation model was used to evaluate the relative importance of Se uptake from dissolved and dietary sources and to explain the interspecies differences in Se bio-

Table 1. Equations describing Se(IV) uptake by *Corbicula fluminea* and *Potamocorbula amurensis* from dissolved sources

Temperature (°C)	Salinity (psu)	Shell size (mm)	Equation	k_u	r^2
C. fluminea					
5	0	19.5 ± 1.1	$I_w = 0.0010 \cdot C_w^{1.00}$	0.0010	0.999
13	0	19.1 ± 1.2	$I_w = 0.0029 \cdot C_w^{1.08}$	0.0029	0.996
21	0	18.9 ± 1.2	$I_w = 0.0040 \cdot C_w^{0.99}$	0.0040	0.999
13	0	19.1 ± 1.2	$I_w = 0.0029 \cdot C_w^{1.08}$	0.0029	0.999
13	4	19.0 ± 1.2	$I_w = 0.0019 \cdot C_w^{1.00}$	0.0019	0.996
13	8	19.0 ± 0.5	$I_w = 0.0025 \cdot C_w^{0.99}$	0.0025	0.999
P. amurensis					
13	4	16.7 ± 1.3	$I_w = 0.0110 \cdot C_w^{0.94}$	0.0110	0.999
13	8	16.7 ± 1.6	$I_w = 0.0084 \cdot C_w^{0.93}$	0.0084	0.998
13	20	16.4 ± 1.6	$I_w = 0.0048 \cdot C_w^{0.93}$	0.0048	0.999
13	8	13.2 ± 0.5	$I_w = 0.0112 \cdot C_w^{0.93}$	0.0112	0.996
13	8	15.7 ± 1.0	$I_w = 0.0091 \cdot C_w^{0.93}$	0.0091	0.995
13	8	18.0 ± 0.5	$I_w = 0.0078 \cdot C_w^{0.93}$	0.0078	0.998
13	8	19.7 ± 0.5	$I_w = 0.0066 \cdot C_w^{0.87}$	0.0066	0.997

[a] Regression equations are based on log-transformed data relating metal influx rate (I_w) to dissolved Se concentration (C_w). Uptake rate constants (k_u) were determined using the methods described in the text.

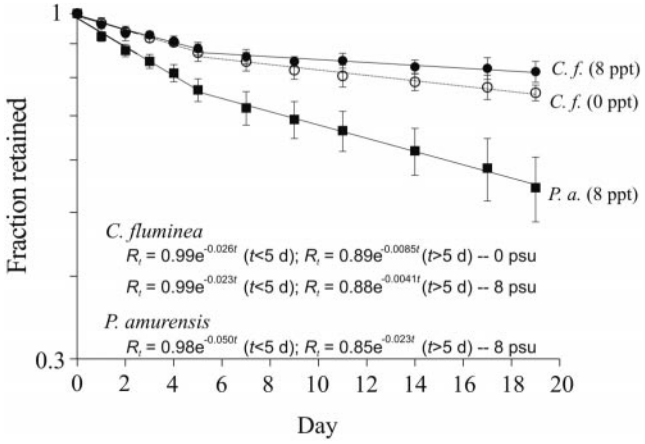

Fig. 2. Retention of bioaccumulated Se in *Corbicula fluminea* (*C.f.*) in media at 0 or 8 psu and *Potamocorbula amurensis* (*P.a.*) in media at 8 psu. The physiological turnover rates of assimilated Se in clam tissues was described by $R_t = R_1 \cdot e^{-ke \cdot t}$ ($t < 5$ d) and $R_2 \cdot e^{-ke \cdot t}$ ($t > 5$ d). The term R_1 represents the fast-exchanging pool and the term R_2 the slow-exchanging one. Error bar indicates the standard deviation ($n = 4$). (See Eqn. 4 for the definition of the parameters.)

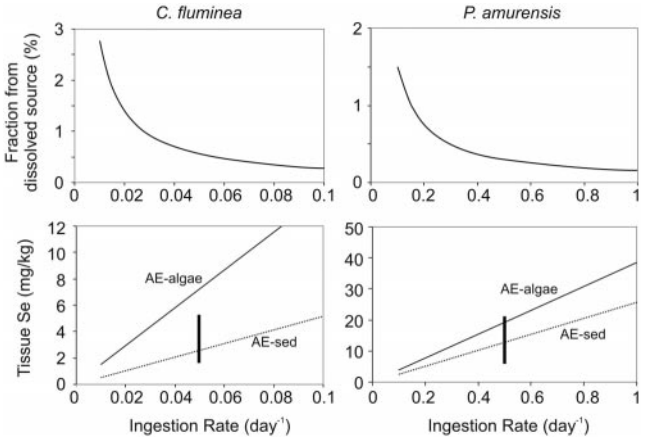

Fig. 3. Fraction of tissue Se obtained from dissolved sources (%) or predicted tissue Se concentration (mg/kg) in *Corbicula fluminea* and *Potamocorbula amurensis* as a function of ingestion rate (IR; /d). Tissue Se was predicted when average diets of the clams were assumed to be algae (solid line) or oxidized sediments (dotted line). Vertical bar represents a range of observed tissue Se concentrations (μg/g) in both clam species from San Francisco Bay, USA [8]. See Table 2 for the physiological and geochemical parameters used for the model estimation. The terms AE-algae and AE-sed indicate assimilation efficiency of Se associated with algae and sediment particles, respectively.

accumulation between *C. fluminea* and *P. amurensis* in North SFB. The biological and geochemical parameters used for the model calculations are summarized in Table 2. The mean Se concentrations in water and suspended particles in North SFB measured in 1996 were used for C_w (0.18 μg/L) and C_f (2.0 μg/g) [8,19]. All dissolved Se was assumed to be selenite. Because the k_u, AE, and k_e values for *C. fluminea* and *P. amurensis* were variable depending on environmental conditions, the mean values determined in the present study were used for model calculation, as noted above. Because published growth rates for these clams are not available, the growth rate of the clams was assumed to be 0.005/d. A range of ingestion rates was used for the model calculation.

The results of the model suggest that the relative contribution (%) of dissolved Se to total Se bioaccumulation in *C. fluminea* and *P. amurensis* would be less than 3% (Fig. 3). The contribution from dissolved sources decreases with ingestion rates. The predicted total concentrations of Se in *P. amurensis* and *C. fluminea* increased with the ingestion rate and were greater with ingestion of algal diets than with oxidized surface sediments (Fig. 3).

Table 2. Various mean parameters used for the bioaccumulation models of *Potamocorbula amurensis* and *Corbicula fluminea* and the ranges of tissue Se concentrations observed for the clams from sites located in North San Francisco Bay, USA[a]

	P. amurensis	*C. fluminea*
Se concn. in water (μg/L)	0.18	0.18
Se concn. in sediment (μg/g)	2.0	2.0
k_u (L/g/d)	0.009	0.0025
AE-sediment (%)	36	29
AE-algae (%)	54	81
k_e (/d)	0.023	0.006
Growth rate constant (/d)	0.005	0.005
Ingestion rate (/d)	0.1–1.0	0.01–0.1
Tissue Se concentrations (mg/kg)	3.9–20.0	1.4–4.8

[a] Linville et al. [8]. AE = assimilation efficiency of Se.

DISCUSSION

Assimilation from dietary source

The high Se AEs in *C. fluminea* and *P. amurensis* observed in the present study are consistent with those in previous studies (see, e.g., [9]), which demonstrated that Se is highly bioavailable from ingested particles to invertebrates. The AEs of Se in algal food for the two clam species were comparable to those reported in previous studies with other clams (50–90% in *M. balthica*, *P. amurensis*, and *Ruditapes philippinarum*), mussels (48–84% in *Mytilus edulis* and *Dreissena polymorpha*), oysters (52–74% in *Crassostrea rivularis* and *Saccostrea glomerata*), bivalve larvae (>97%), marine copepods (73–97%), and barnacles (79%) [9,10,12,20–24]. Similarly, the lower AE of Se from ingested oxic sediment particles also has been reported in other bivalve species, including *M. balthica* (21–27%), *C. rivularis* (26%), and *M. edulis* (14–27%) [24,25]. The reduced bioavailability from ingested inorganic particles compared to algal-rich particles has been reported for other metals in various marine invertebrates [17,26].

The bioavailability of Se in sediment particles could be significantly altered by changes in Se speciation within sediment particles. The present study used bulk oxidized surface sediment for the measurement of Se AE. Schlekat et al. [12] reported that *P. amurensis* assimilated Se from ingested oxic sediment with significantly higher efficiency than from anaerobic sediments. Those authors further demonstrated that chemical or biological reduction of Se(VI) in anoxic sediment to elemental Se dramatically reduced the Se AE in *P. amurensis* to nearly 3%. The chemical species of Se in anaerobic sediments include elemental Se, organoselenium, and selenoanions [12]. Therefore, the bioavailability of Se in contaminated sediment could have significant temporal and spatial variability depending on the redox cycles of Se speciation.

Estuarine invertebrates experience a wide range of salinity and temperature fluctuations. Changes in salinity and temperature may alter various biological (e.g., metabolic rate, water

permeability of gut epithelium, and enzymatic activity) and physicochemical processes (e.g., partitioning of metals to food particles and water viscosity). Very few studies have been conducted to evaluate the influence of salinity or temperature on metal AE [21,26,27]. These studies generally have shown that the effects of salinity and temperature on metal AE were minor, unpredictable, or inconsistent among studies. For example, unpublished data showed that AEs of Cd, Cr, and Zn associated with algal and sediment diets in *C. fluminea* were influenced neither by temperature nor by salinity (B.G. Lee, unpublished data). Consistent with previous studies, temperature did not have a significant influence on Se AEs for either of the clams in any case, and a significant effect of salinity was observed only in a few cases. In contrast, their influences on the dissolved Se uptake rate were much greater, as discussed below.

Uptake from dissolved sources

The Se influx rate constant (k_u) in *C. fluminea* (0.001–0.003 L/g/d) was quite low, and that in *P. amurensis* (0.005–0.011 L/g/d) was within the variability among the values determined for other bivalves, such as *M. balthica*, *M. edulis*, *C. rivularis*, and *D. polymorpha*, in previous studies [9–11,24]. Lee et al. [17] suggested that greater Cd, Cr(VI), and Zn influx in *P. amurensis* than in *M. balthica* was probably caused by differences in the clearance rates of the two bivalves. Similarly, Wang [28] reported that the mean influx rate constant of Cd, Cr(VI), and Se in three bivalves (*Perna viridis*, *Septifer virgatus*, and *R. philippinarum*) was strongly related to the clearance rate of bivalves. Because the clearance rates for *P. amurensis* were approximately 10-fold the rates for *C. fluminea* [29–31], the interspecific difference of dissolved Se uptake between *C. fluminea* and *P. amurensis* could be explained by the differences in clearance rates. The interspecific differences in the metal influx rates also may be related to factors other than clearance rate, including species-specific gill surface area, metabolic rate, or permeability of gill epithelium to dissolved elements [32,33]. However, most of these factors (with the possible exception of the permeability of gill epithelium) could covary with each other and seem to be related to the body size of each animal species.

The increased metal influx rate at higher temperatures often was explained by the increased metabolic rate. The increase in k_u values of Se for *C. fluminea* with a temperature increase from 5°C (0.001 L/g/d) to 21°C (0.0033 L/g/d) corresponds to a Q_{10} value (the factor by which the metabolic rate differs for a temperature interval of 10°C) of two, which is typical for metabolic response of organisms. Similarly, our unpublished results reported that the k_u for Cd, Cr(VI), and Zn uptake by *C. fluminea* increased significantly with water temperature (see above).

The effect of salinity on the Se uptake rate in *P. amurensis* is consistent with the general observation that influx rates of metals in estuarine invertebrates are inversely related to salinity [17,33]. No apparent explanation is available for why Se uptake in *C. flumenia* did not respond to changes in salinity. In general, the influence of salinity variation on bioaccumulation is more complex than the influence of temperature or size, because salinity can affect not only physiological processes of exposed animals but also geochemical partitioning, ionic strength, and speciation of elements [34,35]. The anionic Se used in the present study might not change its speciation over the range of salinity employed. Similarly, Lee et al. [17] reported an influx rate measurement of waterborne Cr(VI), another element that presents in an anionic form and does not change speciation with a change in salinity; the influx rate of Cr(VI) in the bivalves *P. amurensis* and *M. balthica* increased inversely with salinity. These results collectively suggest that in low-salinity waters, processes other than speciation change were responsible for the high influx of both Se(IV) and Cr(VI) in *P. amurensis*. We speculate that a reduced concentration of competitive anions in seawater (e.g., SO_4^{2-}) for binding/transport sites of SeO_3^{2-} on the gill surface and/or increased water permeability in gill epithelium caused by salinity reduction were the probable cause of the increased rate of Se uptake [36]. In fact, approximately 30% more Se adsorbed on the shells at 4 psu than at 20 psu after exposure to waterborne Se, indirectly suggesting that fewer competing anions were present at lower salinity [37]. Alternatively, the greater Se(IV) uptake at lower salinity may be related to a Na-SO_4 transporter [38]. At low salinities, when the organism is hyper-regulating, this transporter may be significantly up-regulated to facilitate Na uptake. In turn, this would result in increased uptake of SO_4 (or Se, assuming transport on the same carrier).

Efflux of tissue Se

Physiological turnover rates of assimilated Se were adequately predicted by a two-compartment loss model with fast- and slow-exchanging pools. This multicompartment loss pattern may reflect the partitioning of Se in the different organs and subcellular pools with different loss kinetics. The biological half-lives ($t_{1/2}$) for Se in the slow-exchanging component of *C. fluminea* (82 d at 0 psu and 169 d at 8 psu) were relatively long compared to *P. amurensis* (30 d) and other bivalves, such as the freshwater mussel *D. polymorpha* (20–27 d) [11] and *M. edulis* (28–40 d) [10], but were comparable to *Mytilus galloprovincialis* (80 d) [39]. The faster turnover rate of Se in *P. amurensis* relative to that in *C. fluminea* might be related to body size. Mean tissue dry weight of *P. amurensis* (0.02 g) was sixfold less than that of *C. fluminea* (0.12 g) in the present study.

Se bioaccumulation model

The results of modeling suggest that dietary uptake is the dominant pathway for Se bioaccumulation in *C. fluminea* and *P. amurensis* over a range of biological and environmental conditions. Similarly, previous studies have reported the dominance of dietary sources for Se bioaccumulation in bivalves [9,10,24] and other aquatic animals [23,40]. For example, using a modeling approach similar to that in the present study, Luoma et al. [9] demonstrated that 99% of the Se bioaccumulation by *M. balthica* in SFB could be explained by the dietary uptake pathway.

Tissue Se concentrations in *P. amurensis* observed in North SFB were the highest among the bivalves and ranged from 3.7 to 20 mg/kg [8]. From the same sampling location, tissue Se concentrations in *C. fluminea* were much lower than those in *P. amurensis* and ranged from 1.4 to 4.8 mg/kg. The present study attempted to explain why these two clam species display different levels of Se bioaccumulation even though they are exposed to similar environmental conditions. Although the influx rate from dissolved Se in *P. amurensis* is approximately three- to fivefold greater than those for *C. fluminea*, uptake from dissolved sources could not explain the difference in Se bioaccumulation between the two clam species, because the uptake from dissolved sources represents less than 3% of total

Se bioaccumulation. Additionally, the rate constants for loss in *P. amurensis* are three- to fivefold greater than those in *C. fluminea*. Therefore, the interspecies difference in Se bioaccumulation between the two clams likely is caused by the differences in Se bioaccumulation from dietary sources.

The parameters required for estimation of dietary Se bioaccumulation are the AE, ingestion rate, and Se concentration in diets. Because Se concentrations in diets are assumed to be the same for both clam species and the difference between AEs for the two clams was less than doubled, it can be determined that the ingestion rate is the only variable that controls Se bioaccumulation from dietary sources. If *P. amurensis* were to ingest 0.15 to 0.85 g oxic sediment/g dry weight/d or 0.1 to 0.5 g algae/g dry weight/d, the clam would achieve the range of Se concentrations observed in the field sites. Similarly, if *C. fluminea* ingested 0.02 to 0.1 g oxic sediment/g dry weight/d or 0.005 to 0.035 g algae/g dry weight/d, tissue Se in the clams would approach the levels observed in field sites. These ingestion rates of sediment and algae in *P. amurensis* are 8-fold and from 15- to 20-fold those in *C. fluminea*, respectively. To our knowledge, no data have been published regarding the ingestion rates of these two clams against which to compare these estimated ingestion rates. The reported clearance rates of *P. amurensis* [31,32] were all within similar ranges (100–600 L/g/d) and are 4- to 100-fold greater than those of *C. fluminea* determined separately in the present study (6–27 L/g/d). The ingestion rate of filter-feeding bivalves is a function of the clearance rate and particle concentrations. Therefore, the greater clearance rate of *P. amurensis* compared to *C. fluminea* could explain, in part, the greater estimated ingestion rate of *P. amurensis* required for explaining the interspecies difference of Se bioaccumulation in North SFB.

The biokinetic model approach can be used not only as a predictive tool for estimating tissue Se bioaccumulation when external environmental factors change but also as a diagnostic tool for understanding the various geochemical and biological processes that affect Se bioaccumulation. The information acquired from the model can be applied to scientific assessment and management of Se-contaminated environments.

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