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Assimilation and retention of selenium and other trace elements from crustacean food by juvenile striped bass (*Morone saxatilis*)

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Abstract

Estimates of the assimilation and retention of trace elements from food by fish are useful for linking toxicity with the biogeochemical cycling of these elements through aquatic food webs. Here we use pulse-chase radiotracer techniques to estimate the assimilation and retention of Se and four trace metals, Ag, Am, Zn, and Cd, by 43- and 88-d-old juvenile striped bass, *Morone saxatilis*, from crustacean food. Brine shrimp nauplii, *Artemia franciscana*, or adult copepods, *Acartia tonsa*, were fed radiolabeled diatoms and then fed to juvenile striped bass. Assimilation efficiencies (AEs ± SD) for 43-d-old fish were 18 ± 2%, 6 ± 1%, 23 ± 4%, 33 ± 3%, and 23 ± 2% for Ag, Am, Cd, Se, and Zn, respectively. For 88-d-old fish, the AEs were 28 ± 1%, 42 ± 5%, and 40 ± 5% for Cd, Se, and Zn, respectively. The higher AEs in the older fish may result from longer gut passage times for larger fish. The 44-d-old fish excreted 5 ± 0.8%, 4 ± 2.0%, 7 ± 0.3%, 9 ± 0.4%, and 1.3 ± 0.9% of the Ag, Am, Cd, Se, and Zn, respectively, they ingested from food per day, whereas the 88-d-old fish excreted 3 ± 1.0%, 8 ± 0.5%, and 3 ± 0.5% of the assimilated Cd, Se, and Zn per day, respectively. Predictions of steady state Se concentrations in juvenile striped bass tissues made using a biokinetic model and the measured AE and efflux rates ranged from 1.8 to 3.0 μg Se g^{-1} dry wt for muscle tissue and 6.8 to 11.6 μg Se g^{-1} dry wt for gut tissue. These predictions agreed well with average values of 2.1 and 13 μg Se g^{-1} dry wt measured independently in North San Francisco Bay, where elevated Se concentrations are of concern. The model results imply that the planktonic food web, including juvenile striped bass, does not transfer Se as efficiently to top consumers as does the benthic food web.

The ability of fish to accumulate trace elements has received increasing attention in the last two decades as evidence of nutritional and toxicological effects mount (Luoma 1983). Se has generated particular interest because of its multiple roles as a potentially limiting nutrient in Se-deplete areas (Stadtman 1974), a toxic element in aquatic ecosystems affected by high Se in agricultural runoff and industrial wastes (Cutter 1989; Presser 1994; Presser et al. 1994), and a potential antagonist to Hg toxicity and accumulation (Wagemann et al. 1998). Along with Hg and Cs, it is also one of the few trace elements subject to biomagnification (e.g., Luoma et al. 1992). Subtle changes in tissue concentrations of Se can have large effects on fish health. There is only a fourfold difference between tissue concentrations that are considered normal and those that can cause toxicity or teratogenesis in some species (Lemly 1998; Coughlin and Velte 1989).

An extensive literature shows that fish and other aquatic organisms obtain much of their body burden of Se and other trace elements from food (reviewed in Langston and Spence 1995; Reinfelder et al. 1998). Se found in tissues of fish is almost entirely derived from diet (Lemly 1982). Many other trace elements, including Cd, Co, Mn, and Zn, are also predominantly derived from ingested food by bivalves and copepods (e.g., Reinfelder and Fisher 1991; Wang et al. 1996; Wang and Fisher 1998*a*) and are readily assimilated from food by fish (e.g., Pentreath 1973*a*,*b*). Clearly, a predictive understanding of the variables affecting accumulation of Se and other trace elements from food by fish is needed to properly evaluate the biotic and ecosystem level effects of trace element contamination, and to accurately assess the viability of mitigating management strategies.

Toxicokinetic models provide a framework for developing such an understanding (Thomann 1981; Landrum et al. 1992). These models make steady state predictions of contaminant tissue concentrations based on several independently estimated parameters: growth, ingestion rate, element concentration in the food, assimilation efficiency for the element, and efflux rate (Table 1). Key to the successful application of the model is the accurate measurement of element assimilation efficiency and first-order efflux constants using radiotracer pulse-chase experiments (Reinfelder et al. 1998). Predictions of trace element concentrations in crustacean zooplankton and bivalves based on such measurements have agreed well with field data (Wang et al. 1996; Fisher et al. 2000; Roditi et al. 2000). Ultimately, by allowing us to couple information on the kinetics of trace element uptake and loss with bioenergetic models of consumption and growth, biokinetic models of trace element uptake may provide an effective framework for relating biological and

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Table 1. Summary of model parameter symbols.

Symbol	Units	Description
AE	%	Assimilation efficiency: the percentage of an ingested element that is assimilated into animal tissues
k_{ef}	d^{-1}	Efflux constant of element obtained from food: fraction lost per day
g	d^{-1}	Growth: instantaneous daily rate
I	g dry wt g^{-1} dry wt d^{-1}	Ingestion rate
Se_X	μg Se g^{-1} dry wt	Concentration of target element per unit dry weight in pool X
CE	%	Conversion efficiency: the percentage of ingested food converted to biomass by fish
A, B, C	%	Estimates of initial element pool sizes in depuration experiments
k_A, k_B, k_C	d^{-1}	Estimates of decay coefficients for element pools A, B, and C

environmental variables to trace element tissue concentrations. In this way, we can assess which trace elements, species, life stages, and environmental conditions are most important from either toxicological, biogeochemical, or nutritional perspectives.

There is a paucity of quantitative estimates of trace element assimilation efficiency and efflux for fish (Reinfelder et al. 1998). Such information on early life history stages would be especially useful because these stages often represent significant population survivorship bottlenecks. Furthermore, the early life history stages of many ecologically and commercially important fish species tend to be associated with coastal and estuarine habitats and are consequently exposed to high environmental contaminant levels during critical developmental periods. In this study, we measured the assimilation and loss of Se and several other trace elements from food by juvenile striped bass, *Morone saxatilis*. Striped bass are an economically and recreationally important anadromous fish that are common residents or immigrants into estuaries and rivers of North America. They are also sensitive to Se toxicity (Coughlin and Velte 1989).

In this study, we first compare trace element assimilation efficiencies and efflux constants measured for these fish using pulse-chase radioisotope experiments to those previously measured for invertebrates. We then determine if our laboratory measurements for Se are relevant to field conditions by comparing our steady state predictions of Se tissue concentrations to field measurements. Finally, we ask whether there is potential for present or future Se toxicity to striped bass based on our findings and whether the planktonic food web is as efficient a pathway for Se transfer as is the benthic pathway.

Table 2. Description of parameters for each experiment.

Variable	Experiment 1	Experiment 2
Age of fish at start (d)	43	88
Food	*Artemia franciscana*	*Acartia tonsa*
No. of fish per replicate	4–5	1
Mean weight of fish (mg dry wt)	11.8±4.7	92.1±13
Mean length of fish (mm)	19.6±2.3	38.5±2.3

Materials and methods

Rearing of fish—Morone saxatilis larvae were obtained from the Dennis Wildlife Center Fish Hatchery in Bonneau, South Carolina, as 1–2-d-old hatchlings and reared at Flax Pond Marine Laboratory (Crane Neck, New York) in recirculating water tables. Rearing conditions are described elsewhere (Brown et al. 1998). Briefly, the larvae were maintained in freshwater that had been passed through an ion exchange column and then hardened to >250 mg HCO_3 L^{-1} by passing the water over crushed dolomite. Larvae were kept in indirect light and darkened buckets that were continuously flushed with water, except during feeding, from the recirculating water table. Temperatures ranged from 19 to 22°C. Freshly hatched *Artemia franciscana* larvae (Argent Chemical, Silver grade) were used as forage throughout the experiment. Experiments were conducted on the fish when they were 43 and 88 d old (Table 2).

Experimental protocol—Seawater collected from the Flax Pond laboratory (salinity = 26) was autoclaved and amended with f/2 nutrients excluding Cu, Zn, and EDTA (ethylenediamine tetraacetic acid) (Guillard and Ryther 1962). This water was then filtered through a sterile 0.2-μm polycarbonate filter and corrected to pH 8.0 with NaOH, and 800 ml was dispensed into each of two 1-liter flasks. To one flask we added 9.3 kBq of ^{110m}Ag in 3 N HCl and 37 kBq of ^{241}Am in 6 N HNO_3. Another set of flasks received 74 kBq of ^{65}Zn in 0.5 N HCl, 37 kBq of ^{75}Se (as selenite) in DI, and 222 kBq of ^{109}Cd in 0.1 N HCl. In the second set of experiments, 148 kBq of ^{109}Cd was added, while the amounts of ^{65}Zn and ^{75}Se added were the same as in the first experiment. The corresponding added concentrations in the first experiment were 1.3 nM Ag, 3.9 nM Am, 11.6 nM Cd, 0.42 nM Se, and 96 pM Zn; in the second experiment, they were 1.4 nM Ag, 3.9 nM Am, 4.9 nM Cd, 0.54 nM Se, and 0.12 nM Zn. The algal media with added radiolabeled trace elements were allowed to equilibrate for 24 h and then were inoculated (Fisher and Wente 1993) with log-phase cells of the diatom, *Thalassiosira pseudonana* (clone 3H), to give an initial density of 1×10^5 cells ml^{-1}. The cells were allowed to grow for 2 d to densities of 5–6 $\times$ 10^{-5} cells ml^{-1}.

Two sets of feeding experiments were conducted, one using *Artemia franciscana* larvae and the other using adults of

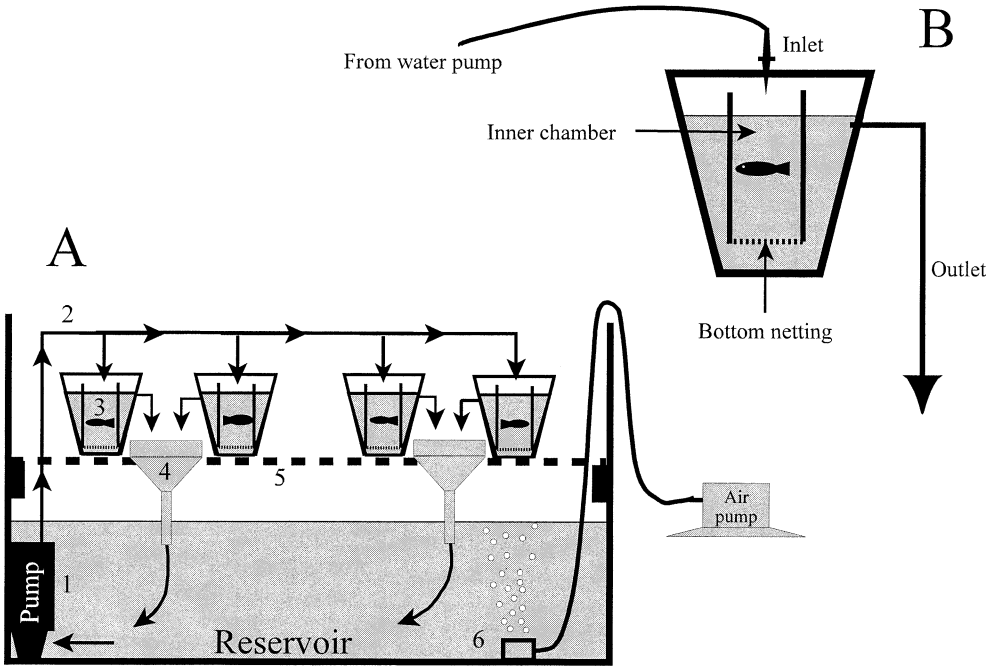

Fig. 1. Illustration of setup for the depuration experiments. (A) The experimental chambers. (B) The system for recirculating water through the experiments. 1, Powerhead water pump; 2, Tygon tubing for transferring water from the pump to the experimental chambers; 3, experimental chamber; 4, funnel fitted with 60-μm mesh Nitex netting; 5, plastic grating; 6, airstone.

the copepod, *Acartia tonsa.* The *Artemia* were hatched from cysts in the lab from the same stocks used to feed the fish. The copepods were handpicked from zooplankton net samples (72 μm) taken from Stony Brook harbor. Approximately 2,000 1-d-old *Artemia* larvae or 800 *A. tonsa* adults were added to the flasks containing the labeled algae and allowed to feed for 16–24 h. Just prior to feeding these labeled zooplankton to the fish, the *A. franciscana* and *A. tonsa* were concentrated by gently pouring the contents of the flasks through a 100-μm Nitex mesh and immediately resuspending in 100 ml of filtered seawater.

The experimental apparatus for exposing the juvenile fish consisted of an 80-liter tank containing 20 liters of continuously recycled and bubbled 0.2-μm-filtered seawater that served to dilute radioisotope released during depuration, thus greatly reducing recycling (Fig. 1). Suspended above this filtered seawater reservoir on a plastic grating were chambers consisting of an outer 120-ml plastic container and an inner plastic cylinder closed at the top and bottom with fiberglass netting within which the fish were enclosed. This design further reduced recycling of radioisotope by allowing feces to fall through the netting and away from the fish. The chambers were cleaned prior to every time point by repeatedly decanting and flushing the chambers with fresh filtered seawater. This procedure greatly reduced mortality from stress associated with removing fish completely from the water. At most, one fish from each container died over the 3-week experiments with the small fish. There was no mortality in the experiments with larger fish. Between counts, water from the 20-liter reservoir was continuously pumped into the central cylinder of each chamber. Fish were fed once daily by ceasing the water flow and adding freshly hatched *Artemia* larvae via pipette directly to the experimental chambers. After 1 h, flow was resumed and the remaining *Artemia* were flushed out of the chambers and captured on 60-μm nets before the water was returned to the reservoir. These nets were cleaned daily. There were four replicate chambers for each treatment.

An hour before the start of experiments, the netted plastic cylinders with fish were quickly removed from the experimental apparatus and placed in plastic sample containers containing 100 ml of filtered Flax Pond water. Fish were allowed to acclimate for an hour to this setting. To initiate the experiment, the solution with concentrated *Artemia* and *Acartia* was first agitated to distribute the crustaceans randomly, after which a 20-ml aliquot was filtered onto a 100-μm mesh Nitex net, rinsed three times with filtered Flax Pond seawater, and then rinsed into the feeding containers. Fish were allowed to feed until they had eliminated >95% of their prey within the netted plastic cylinders (i.e., 15 min for the *Artemia*/small fish experiment and 30 min for the *Acartia*/large fish experiment). After feeding, the cylinders containing the fish were removed from the feeding solution, passed quickly through three rinses of 0.2-μm-filtered Flax Pond water, and deposited into depuration containers containing filtered seawater for immediate gamma counting (*see below*). Immediately after the first gamma count, the fish were fed an excess of unlabeled *Artemia* for an hour. Previous experience showed that the fish filled their guts in this amount of time.

At each time point, feces were collected from the depuration chamber just prior to gamma counting. The chambers

were decanted and flushed with 0.2-μm-filtered Flax Pond water repeatedly until little particulate material was remaining. Any remaining particles were removed by pipette and added to the decanted material. The decanted fluid was then filtered through a 0.2-μm polycarbonate membrane, and the radioactivity of the filter was determined. If a fish had died during the prior sampling interval, it was also removed and analyzed. At the end of the depuration experiment, fish were sacrificed and dissected, and their radioactivity was assayed. Head, gut, and tail regions were separated and dried to constant weight at 60°C. The dried tissues were then weighed and set aside for gamma counting.

Gamma radiation assays—Radioactivity associated with fish was determined in a NaI(Tl) deep-well gamma detector (Canberra). Fish were counted in their depuration chambers to reduce stress. To limit errors in gamma counting due to sample geometry relative to the detector, vertical movement of the fish within the chambers was limited by decanting all but the bottom 1 cm of water from the depuration chambers. X-ray emissions of ^{109}Cd were assayed at 22–26 keV, whereas gamma emissions of ^{241}Am, ^{75}Se, and ^{65}Zn were assayed at 60 keV, 279 keV, and 1,115 keV, respectively. Gamma emissions of ^{110m}Ag were at 657 keV and 884 keV. Counting times were adjusted to yield <5% analytical error or to a maximum time of 15 min. All counts were corrected for blanks and spillover among isotopes. Radioactivity associated with feces, dead fish, and fish parts were counted with an intercalibrated NaI(Tl) well detector (Wallac-Pharmacia Compugamma) fitted with an autosampler.

Statistical analysis—Gut passage time (GPT) was operationally defined as the time needed to clear the guts of 95% of the ingested, unassimilated radioactivity. To determine this value, the radioactivity accumulated through time in fecal matter was regressed against time using the model $y = y_0 + a(1 - e^{-bt}) + c{\cdot}t$. The linear term $c{\cdot}t$ accounts for the continuing presence of some radioactivity in fecal material after the main pulse of radioactivity has ended. This radioactivity probably reflects a small amount of re-adsorption of excreted radiotracer onto fecal particles. Consequently, only the term $y_0 + a(1 - e^{-bt})$ is taken to reflect the defecation of the radiotracer pulse. The intercept, y_0, accounts for the delay between feeding and the initial production of radioactive feces. The equation predicts an asymptotic approach of y over time to the maximum ($y_0 + a$). The GPT is thus the time needed for y to reach 95% of this value. By substituting $GPT = 0.95(a + y_0)$ for y, we find that $GPT = \ln[0.05(1 + y_0a^{-1})]b^{-1}$. Each replicate was analyzed separately, and the mean and standard deviation were determined from the replicate values.

Radioactivity in the depurating fish was regressed against time to determine the assimilation efficiencies (AE) and efflux constants for the isotopes assimilated from food (k_{ef}). Either a two- or three-pool exponential decay model was used. In both cases, the assimilation efficiency is presumed equivalent to the size of the pool with the longest turnover time (*see below*). The decay coefficient for that pool is presumed to approximate the efflux constant. Data from each replicate were analyzed separately, and the estimates of AE and k_{ef} were averaged.

Table 3. Parameters used to predict steady state Se tissue concentrations for juvenile striped bass feeding on San Francisco Bay zooplankton under low- and high-accumulation scenarios.

Parameter	Low	High
AE (%)	42	
k (% d^{-1})	8	
$Se_{Muscle} : Se_{Body}$	0.65	
$Se_{Liver} : Se_{Body}$	2.53	
CE (%)	30	
SE_{zoop} (μg Se g^{-1} dry wt)	5	7
g (% d^{-1})	5	15
IR (g g^{-1} d^{-1})	0.17	0.5

Model validation—Estimates of steady state Se tissue concentration in juvenile striped bass were made according to the equation $Se_{ss} = AE{\cdot}IR{\cdot}Se_f/(g + k_{ef})$, where g is the dry weight–specific growth rate (d^{-1}), Se_{ss} is the steady state Se concentration in fish tissues (g Se g^{-1} dry wt), Se_f is the Se concentration in food (g Se g^{-1} dry wt), and IR is the ingestion rate (g dry wt g^{-1} dry wt d^{-1}). This equation ignores the effect of Se absorbed directly from the dissolved phase, which is generally negligible in marine animals (Wang et al. 1996; Wang and Fisher 1998*a*). The values used in the calculation are presented in Table 3. AE and k_{ef} values for the large fish were used. A minimum Se_f was assumed to be equivalent to 5 μg Se g^{-1} dry wt based on measurements of mixed zooplankton communities (73–3,000-μm size fraction) from Suisun Bay, San Francisco Bay (M. Doblin et al. pers. comm.). Stable isotope analyses indicate that juvenile striped bass in San Francisco Bay feed heavily on mysid shrimp (R. Stewart unpubl. data). There are no extant data on Se contents in mysids in San Francisco Bay, but recent work indicates that mysids should possess Se tissue concentrations, at most, 1.4-fold higher than their zooplankton prey based on measured AE and k_{ef} (C. Schlekat pers. comm.). Consequently, we used 7 μg Se g^{-1} dry wt as a maximum estimate of Se_f.

A range of ingestion rates was calculated assuming high and low accumulation scenarios. Maximal weight-specific growth rates for young (0–28 d) juvenile striped bass range from 0.12 to 0.2 d^{-1} depending on latitude of the source population and temperature (Conover et al. 1997). For the high-growth scenario, we adopted 0.15 d^{-1} because it is typical of maximal growth rates for striped bass larvae and juveniles in low-midtemperate waters at 20–25°C (Conover et al. 1997; Brown et al. 1998). For the low-growth scenario, we arbitrarily chose a rate ⅓ of the high-growth scenario. To calculate ingestion (IR), we assumed a gross conversion efficiency (CE, the ratio between biomass production and ingestion) of 0.30. This CE was determined by fitting the University of Wisconsin fish bioenergetics model (S. W. Hewitt and B. L. Johnson, University of Wisconsin Sea Grant Institute) to juvenile wet weights for fish sampled from San Francisco Bay in September (*see below*) assuming the fish were hatched in mid-March (Heubach et al. 1963)

and using seasonal temperature data for Alameda, California (NOAA, National Oceanic Data Center), and bioenergetic parameters from Moore (1988). We can calculate IR under the fast and slow growth scenarios according to the steady state equation IR = g/CE. We estimated Se concentrations in gut and tail portions of the fish by multiplying the whole-fish Se tissue concentration by the average relative tissue concentration of Se in the gut and tail.

High (high IR, high Se_f) and low (low IR, low Se_f) predictions of Se tissue concentrations in young juvenile striped bass were compared to concentrations measured in liver and muscle of 13 juvenile (~6 months) striped bass collected from Suisun Bay on 30 September 1999 and in 15 adult striped bass collected from San Pablo Bay (n = 7, 7 December 1999) and Suisun Bay (n = 8, 17 December 1999). Analysis of Se was by hydride generation after total digestion in concentrated nitric and perchloric acids at 200°C and reconstitution in hydrochloric acid. Recoveries of total Se in reference materials were within the accepted range for the materials (CRC Dorm2: 1.4 ± 0.1 [measured, n = 6], 1.4 ± 0.1 [accepted]; CRC Dolt2: 5.8 ± 0.3 [measured, n = 7], 6.1 ± 0.5 [accepted]; CRC Tort2: 5.2 ± 0.3 [measured, n = 6], 5.6 ± 0.7 [accepted]).

Results

Following consumption of radiolabeled *Artemia franciscana*, the radioactivity in *M. saxatilis* was 10.6 Bq ^{241}Am, 6.7 Bq ^{110m}Ag, 47.5 Bq ^{109}Cd, 65.2 Bq ^{75}Se, and 53.6 Bq ^{65}Zn per experimental replicate. In the experiment with *Acartia tonsa*, each fish ingested 0.9 Bq ^{109}Cd, 10.5 Bq ^{75}Se, and 4.7 Bq ^{65}Zn. The experiments did not expose the fish to a large pulse of trace elements relative to background. In the first experiment, the uptake of label and carrier by the fish increased their overall tissue concentration relative to dry weight by 6 ng g^{-1} dry wt for Cd, 3 ng g^{-1} dry wt for Zn, and <2 ng g^{-1} dry wt for Se, Ag, and Am. In the second experiment, the increase for all trace elements was less than 1 ng g^{-1} dry wt. For Cd, Zn, Se, and Ag, these values are substantially less than typical tissue residues observed for fish in undisturbed environments (Kennish 1996). Consequently, we believe our results best represent trace element processing by juvenile striped bass when ambient trace element concentrations are near natural background levels. Se in the zooplankton of marine portions of San Francisco Bay is only two to three times more concentrated than in the zooplankton of other uncontaminated environments (M. Doblin unpubl. data).

All radioisotopes in the fish declined with time (Figs. 2, 3). After an initial period of minimal loss of radiotracer, two distinct phases of depuration were apparent. The first was a period of rapid decline that corresponded to the production of highly radioactive feces. We interpret this phase as primarily due to defecation of unassimilated radioisotope. After 24–36 h, radioactivity in feces was at low or background concentrations, and loss of radioisotope from the fish slowed significantly. The loss of radioactivity after 36 h is presumed to correspond to excretion of assimilated radioisotope. Loss of radioisotope from fish in both egestion and excretion

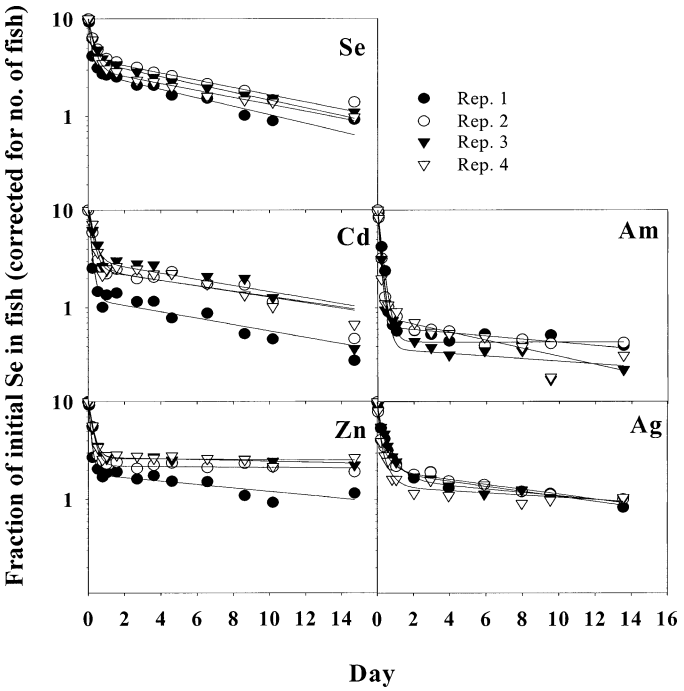

Fig. 2. Loss of ^{75}Se, ^{109}Cd, ^{65}Zn, ^{241}Am, and ^{110m}Ag from 43-d-old *M. saxatilis*-fed radiolabeled *Artemia franciscana*. Each point represents a single experimental chamber holding two to five individuals. Radioactivity is normalized to the number of fish alive in the chambers at the time of measurement. The lines represent best fit exponential decay models of two or three compartments (*see text*). Regression lines are fitted to each replicate separately.

phases approximately followed first-order decay kinetics (Figs. 2, 3). Two-pool exponential decay models were sufficient to explain >90% of the observed variance (adjusted r^2) in radiotracer loss from the fish for ^{75}Se (98–99%), ^{65}Zn (92–98%), ^{109}Cd (90–96%), and ^{241}Am (95 to >99%) (Table 4). However, a three-pool model was necessary to explain the data for ^{110m}Ag (adjusted r^2 > 99%). Two of the pools in the Ag model displayed turnover times <1 d (i.e., efflux rate constants of >100% d^{-1}), so we presume these two pools correspond to some partitioning of unassimilated material. Consequently, the slow third pool was used to estimate AE and k_{ef} for Ag.

Se was the element most efficiently assimilated from food by both small and large fish, although the difference from Cd was borderline insignificant for the small fish (P = 0.06, two-tailed t-test), and the difference from Zn was insignificant in the large fish (P = 0.79, two-tailed t-test) (Table 4). Am was the least efficiently assimilated element in all cases. Ag was assimilated at efficiencies greater than Am and less than Cd and Zn, but the latter differences were not significant (P = 0.24 and 0.06, respectively). The ranking of elements with regard to AE was Se > Zn = Cd > Ag > Am for the small fish and Se = Zn > Cd for the large fish. Se (P = 0.05, two-tailed t-test) and Zn (P = 0.01) were assimilated more efficiently by the large fish, but Cd assimilation did not differ appreciably between the two sizes of fish.

There were systematic differences in the rate of efflux (k_{ef}) for the elements studied. With the exception of Am, for

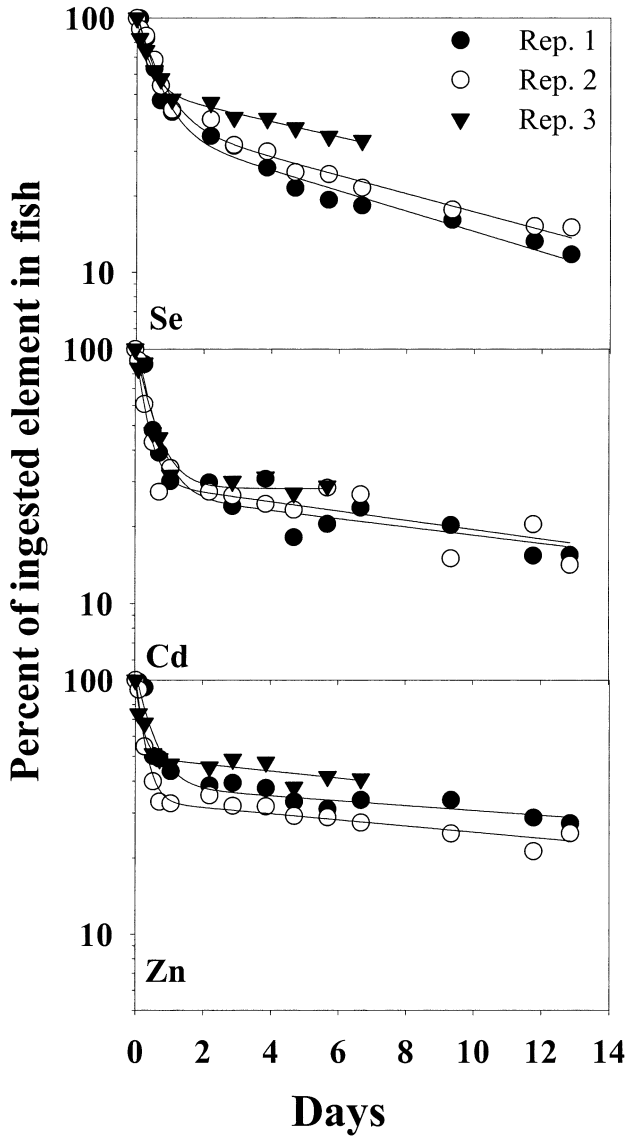

Fig. 3. Loss of ^{75}Se, ^{109}Cd, and ^{65}Zn from 88-d-old *M. saxatilis* fed radiolabeled *Acartia tonsa.* Each point represents a single experimental chamber holding one individual. The lines represent best fit exponential decay models of two compartments (*see text*). The lines are fitted to each replicate separately.

which estimates of k_{ef} were extremely variable, efflux rates were significantly higher for Se than for the other elements in both large and small fish (Table 4). Between 8 and 10% of the assimilated Se was lost per day. In contrast, Zn was always retained the most with loss rates averaging 1.5 and 2.5% d^{-1} over the two experiments. Values for Am, Ag, and Cd tended to be intermediate between these extremes. Small fish excreted Cd at a twofold greater rate than did large fish, whereas Se and Zn efflux rates were not distinguishable between large and small fish.

The model used to fit the cumulative fecal matter production data explained >96% of the variance in every case. Estimates of GPT were <24 h for the small fish and ranged from 11 h for Am to 22.5 h for Cd (Table 5). The Se and Zn GPTs were 17 h for the small fish, but increased to 29 h and 35 h, respectively, for the large fish. The GPT for Cd was actually shorter for the large fish than the small fish, but not significantly so because of the wide variability of the GPT estimates for Cd. GPT could predict AE effectively when data for all elements and sizes were combined (Fig. 4; model: AE = y_0 + b log(GPT), adjusted r^2 = 0.61, P = 0.01).

Cd, Se, and Zn differed markedly in their distributions (relative to the whole body) within the fish at the end of the depuration experiment (Fig. 5). Of the Cd in the fish, 70% was located in the tail, 20% in the gut, and 10% in the head. Se and Zn were both primarily associated with the head (55–63%), with about 33–34% being in the tail. Only 4% of the Zn and 11% of the Se was found in the gut. Although the gut was not important in the inventory of the elements we studied, trace element concentrations were considerably greater there. Cd and Se were 4.4 and 2.5 times more concentrated in the gut on a dry weight basis than in the body as a whole. In other ways, concentrations of Se and Cd differed among body parts. Se was 1.2 times more concentrated in the head than in the whole body, whereas the Cd concentration in the head was only 18% of that in the whole body. The reverse was true of concentrations of these elements in the tail, where Cd was more concentrated (1.3×) and Se less concentrated (0.6×) than in the body. Zn was more evenly distributed throughout the body than were the other two elements. Moreover, Zn was unique in being more concentrated in the head (1.5×) than in the tail (0.6×) or gut (0.8×). Insufficient Am and Ag was left in the fish at the end of the depuration phase to determine the tissue distributions of those metals.

Steady state concentrations of Se in striped bass gut and tail tissue predicted with the kinetic model under slow and fast growth scenarios bracketed the mean values measured independently for liver and muscle tissue from striped bass captured from San Francisco Bay (Fig. 6). Concentrations of Se in tail tissues of juvenile striped bass surviving on a diet of zooplankton were predicted to vary from 1.8 to 3.0 μg Se g^{-1} dry wt as growth increased from 0.05 d^{-1} to 0.15 d^{-1} and ingestion increased from 0.17 to 0.5 g dry wt g^{-1} dry wt d^{-1}. Predicted concentrations in gut tissues over the same range in growth and ingestion rate ranged from 6.8 to 11.6 μg Se g^{-1} dry wt. Se tissue concentrations in San Francisco Bay juvenile striped bass muscle and liver tissue averaged 2.1 and 13 μg Se g^{-1} dry wt, respectively.

Sensitivity of Se tissue concentration predictions to variability in CE, AE, IR, and Se_f was deemed unnecessary because all of these predictors are directly or inversely proportional to the predictions. However, the predictions are mildly nonlinearly related to k_{ef} in a manner dependent on the value of g. Under the slow-growth scenario, when g = 5% d^{-1}, a 10% increase or decrease in k_{ef} results in a 6% decrease or a 7% increase in Se_{ss}, respectively. Under the fast-growth scenario, when g = 15% d^{-1}, the same numbers are only 3.5 and 3.8%.

Discussion

The relative differences observed in the assimilation and retention of the different trace elements agree well with what

Table 4. Mean depuration model parameters. Data from each replicate were fit to either a two- or three-pool first-order exponential decay model. A and B represent percentage of ingested radiotracer in pools primarily associated with the unassimilated material in the gut and subject to fast turnover through defecation. The constants K_A and k_B are the first-order decay constants for those pools. C is the percentage of ingested material that is assimilated (AE), and k_c is equivalent to the efflux constant (k_{ef}). $Tb_{1/2}$ is the biological half-life of assimilated elements. Values represent the means of regression coefficients from four (small fish) or three (large fish) replicates ± 1 SE.

Fish	Element	A (%)	k_A (d^{-1})	B (%)	k_B (d^{-1})	C (%)	k_C (d^{-1})	$Tb_{1/2}$ (d)
Small	Ag	44±6	8.9±1.0	39±6	2.1±0.2	18±1.5	0.05±0.008	13.9
Small	Am	94±1	5.7±0.8			6±1	0.04±0.02	17.3
Small	Cd	77±4	3.5±0.6			23±4	0.07±0.003	9.9
Small	Se	67±3	3.7±0.6			33±3	0.09±0.004	7.3
Small	Zn	77±2	4.3±0.7			23±2	0.013±0.009	53.3
Large	Cd	72±1	2.2±0.4			28±1	0.03±0.01	23.1
Large	Se	58±5	2.0±0.5			42±5	0.08±0.005	8.7
Large	Zn	60±5	3.6±1.0			40±5	0.03±0.004	23.1

has been observed in previous studies on two species of silversides (Reinfelder and Fisher 1994). However, the absolute value for Se AE was slightly higher than measured for *Menidia* feeding on copepods (33–40% compared to 29%), and AEs for Zn and Cd were much higher in the present study (23–48% and 23–40% compared to 6 and 3%, respectively). Although assimilation of Ag and Am have not been studied in fish per se, the lower assimilation of these elements, and Am in particular, agrees well with studies of assimilation by benthic bivalves feeding on phytoplankton and radiolabeled seston (Wang et al. 1996; Roditi and Fisher 1999) and for copepods feeding on phytoplankton (Wang and Fisher 1998*a*). The observed ranking of metals in terms of assimilability is consistent with the findings of Reinfelder and Fisher (1991), which indicated that AE for an element is directly related to the tendency for that element to be associated with the cytoplasm of cells.

The difference in AEs observed for Zn, Cd, and Se in this study and that of Reinfelder and Fisher (1994) probably stem from differences in the partitioning of these elements into dissolved internal pools and exoskeleton of the crustaceans. To mimic the condition of high particle load prevalent in estuaries, suspended particle concentrations during the labeling of the crustaceans were much higher in our experiments than in the *Menidia* experiments. Concentrations of algae were approximately five- to sixfold higher in our experiments (5×10^5 cells ml^{-1} or 10 mg dry wt L^{-1} compared to 1×10^5 cells ml^{-1} or 2 mg dry wt L^{-1}) at the initiation of feeding. Consequently, the fraction of total Zn and Cd on algal cells in the suspensions used to label the crustaceans was about 48–65% and 13–15%, respectively (compared to 24 and 1%). Thus, in our experiments, the direct adsorption of these metals onto external exoskeletons was probably reduced significantly, whereas the uptake of the metals internally by ingestion of algae was probably enhanced. We believe the combined effect was to decrease the fraction of accumulated Zn and Cd that was adsorbed onto the exoskeleton and, therefore, increase the assimilability of these metals from crustaceans by the fish. Se, by contrast, was little affected by particle loads because it does not adsorb onto cell walls or exoskeletons in seawater but is actively incorporated into cytoplasm (Fisher and Reinfelder 1991). The differences in AE between our study and that of Reinfelder and Fisher (1994) indicate how high particle loads in estuaries can alter not only the gross flux of trace elements into the food web, but the pathways of that flux and the

Table 5. Gut passage times (GPTs) for the five trace elements in the experiments with small and large fish. GPT is equivalent to the time needed for 95% of the defecation to take place. Cumulative radioactivity collected as feces was regressed against time according to the model $y = y_0 + a(1 - e^{-bt}) + ct$. GPT was calculated from the regression coefficients as $\ln[0.05(1 + y_0\ a)]/-b$ (*see text*). Values are the means of four groups of four to five fish for the small fish experiment and three fish for the large fish experiment ±1 SD. nd, not determined.

	Gut passage time (h)	
Element	Small fish	Large fish
Ag	13.0±3.6	nd
Am	11.1±0.7	nd
Cd	22.5±4.5	14.0±2.9
Se	17.0±2.1	28.6±5.2
Zn	17.0±2.0	39.8±3.1

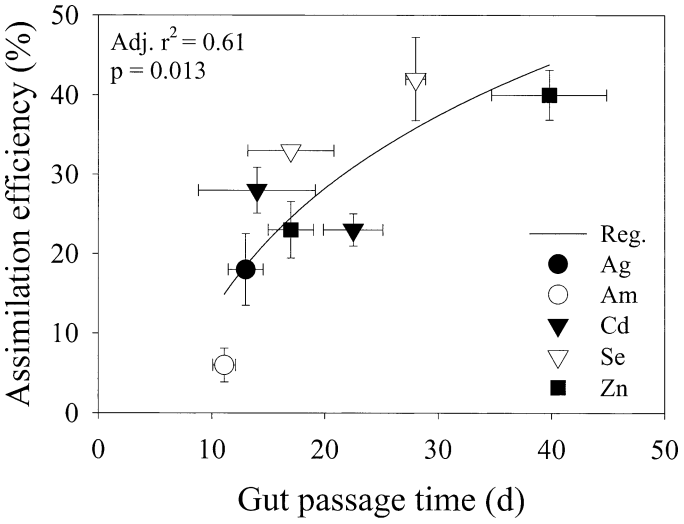

Fig. 4. Correlation between gut passage time (GPT) and AE for all ages of fish and all elements. The regression line represents the equation $AE = a(1 - e^{-b \cdot GPT})$. Error bars are ±1 SD.

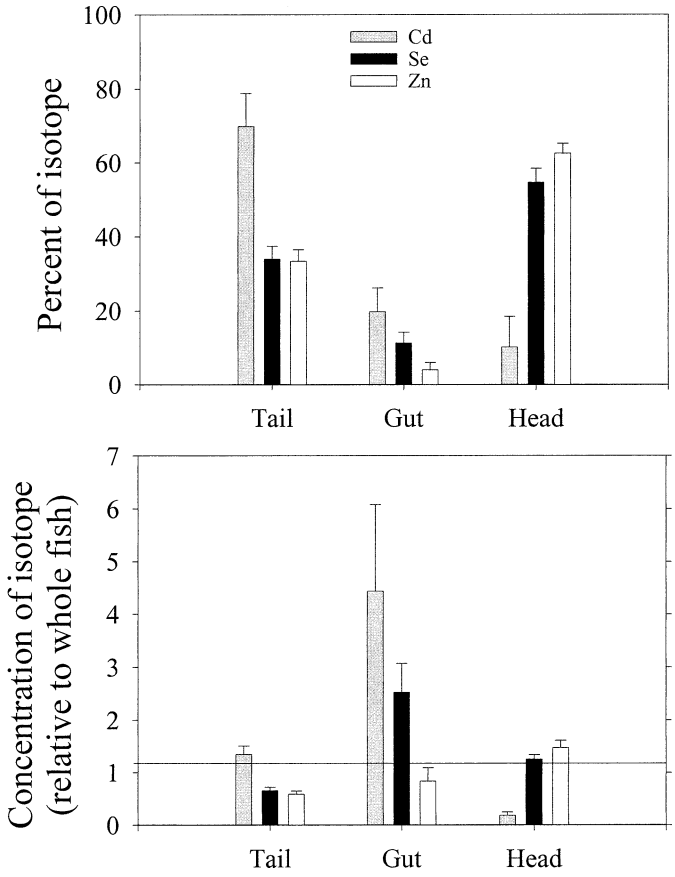

Fig. 5. Distribution of ^{75}Se, ^{109}Cd, and ^{65}Zn within 43-d-old *M. saxatilis* fed radiolabeled *Artemia franciscana.* The top panel represents the fraction of radioactivity associated with each radioisotope in each body part. The lower panel shows the concentration of radioactivity associated with each isotope relative to dry weight. Bars are the means of 14–15 fish. Error bars are ±1 SD.

consequent efficiency of transport of trace elements up food webs.

The ranking of AEs for Am, Ag, Cd, Se, and Zn for striped bass were almost identical to those observed for bivalves and copepods, whereas the absolute values were either comparable or somewhat lower. They seem particularly low relative to estimates of AE for several marine bivalves fed on pure algae, which ranged from 3 to 40% for Am, 13 to 44% for Ag, 37 to 83% for Cd, 70 to 92% for Se, and 62 to 73% for Zn (Reinfelder et al. 1997). However, the values for *Mytilus edulis* fed on radiolabeled seston were more comparable to our results: 4–34% for Ag, 11–40% for Cd, 15–72% for Se, and 16–48% for Zn (Wang et al. 1996). By comparison, AEs of Ag, Cd, Se, and Zn for the copepod *Temora longicornis* fed on algae were 13, 35, 59, and 61%, respectively.

The efflux rates of metals from the juvenile striped bass were generally higher than those from various bivalve species but lower than from marine copepods. The k_{ef}s in bivalves for a range of elements generally vary between 1 and 5% d^{-1} (Fisher et al. 1996; Reinfelder et al. 1997; Roditi and Fisher 1999). By contrast, the k_{ef}s in juvenile striped bass ranged almost sevenfold between Zn and Se, and all

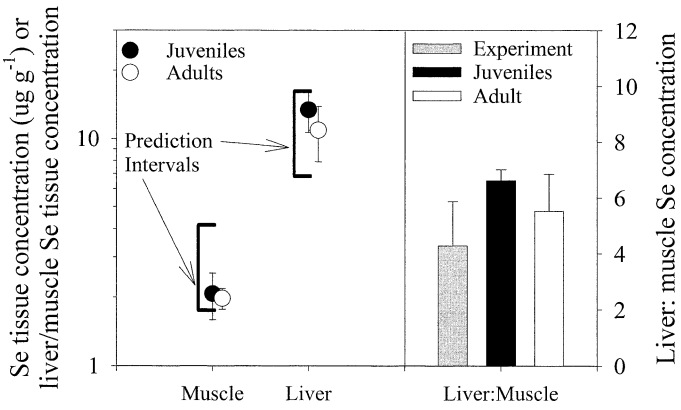

Fig. 6. Comparison of observed and model-predicted Se concentrations in *M. saxatilis* tissues. On the left side, brackets span the range between maximum and minimum predictions of Se tissue concentrations (*see text*). Circles are observed values for 13 juvenile fish and 15 adult fish collected from northern San Francisco Bay. On the right side, bars represent ratios of Se concentrations in different tissues in the experiments and in the field-collected samples. Error bars are ±1 SD.

the values except for Zn exceed 3% d^{-1}. Se in particular was excreted 2–9 times faster by *Morone saxatilis* juveniles than by *Mytilus edulis* (2–5% d^{-1}), *Dreissena polymorpha* (2% d^{-1}), *Macoma balthica* (3% d^{-1}), and *Mercenaria mercenaria* (1% d^{-1}). Ag, Am, and, in particular, Cd also exhibited somewhat higher rates of excretion than typically observed for these elements in bivalves. The k_{ef}s for diverse metals in marine copepods range from 5 to 38% d^{-1}, with values lowest for Zn (5–9% d^{-1}) and typically about 15–20% d^{-1} for Se, Cd, and Ag (Wang and Fisher 1998*b*).

Of the three elements for which we mapped distributions, the two that were most closely associated with gut tissues (Cd and Se) also exhibited faster excretion rates. By contrast, Zn was actually less concentrated in the gut region than elsewhere and was at the same time characterized by barely detectable k_{ef} values. High concentrations of Cd and Se in the gut and high excretion rates of these elements may indicate that the fish were actively detoxifying and excreting these elements. Thus, the distinction between the excretion rates for these elements in fish and bivalves may reflect differences in the efficiency and mechanism of detoxification. Alternatively, these elements, especially Se, may actually be preferentially associated with compounds that are involved in biochemical processes in the liver. Se is a required element in certain enzymes (e.g., glutathione peroxidase) that help catalyze the breakdown of superoxide radicals (Stadtman 1974). It can also immobilize toxic metals such as Hg and Cd into inactive inorganic granules (Martoja and Berry 1980).

Field observations of Se in 6-month-old juvenile striped bass tissues were within the range of predictions for 2-month-old juveniles using kinetic modeling (Fig. 6). This result concurs with those for other organisms, suggesting that biokinetic models can predict trace element concentrations in animal tissues on a site-specific basis to within a narrow concentration range. The observed Se tissue concentrations in muscle of striped bass juveniles and adults are

less than those that have led to lowered reproductive performance in adult *M. saxatilis* (~3 μg g^{-1} dry wt in muscle tissue: Coughlin and Velte 1989). However, the observed values are within 50% of potentially toxic values, and the maximum predictions actually exceed the 3 μg g^{-1} threshold. Zooplankton Se tissue concentrations vary considerably over spatial and temporal time scales in San Francisco Bay (D. Purkerson et al. pers. comm.), possibly in response to changes in dissolved Se concentration or speciation, or shifting phytoplankton species composition and abundance. Moreover, growth and ingestion of larval and juvenile striped bass are likely to vary spatially and through time in a way that may affect Se tissue concentrations in these fish. A doubling of food consumption between a low-growth and a high-growth year has the potential to increase Se concentrations in juvenile striped bass muscle tissue by 88%, presuming that conversion efficiency and Se food content remain constant. Consequently, there is a significant possibility of short-term or localized toxicity to larval *M. saxatilis* in this estuary.

Our results for Se assimilation and retention by fish suggest a greater importance of benthic clams over planktonic organisms in the transport of Se, and perhaps other trace elements, to the top levels of the estuarine food web. Se is predicted to biomagnify only marginally as it progresses up the estuarine planktonic food chain from algae to crustaceans and then to striped bass larvae. The zooplankton on which the fish fed in these experiments, although efficient at assimilating Se from food, excrete 16–19% of their Se d^{-1} (Wang and Fisher 1998*b*). Measurements of Se accumulation by mysids fed crustacean zooplankton suggest that mysids concentrate Se by a factor of 1.1- to 1.4-fold more than their prey. Consequently, Se is not much more concentrated in the zooplankton or mysid prey of these fish than in the phytoplankton at the base of the food chain. When fish are growing quickly and eating a large amount, Se is predicted to concentrate in fish tissues only 1.4-fold above the concentrations in their crustacean prey. Under the opposite scenario Se concentration is actually predicted to be less than that of their food. The lack of a strong tendency for Se to be biomagnified by these fish is a result of their efficient growth, high excretion rates, and, to a lesser degree, their relatively low AEs.

By contrast, Se is more concentrated in the tissues of many filter-feeding bivalves than in their algal prey (Reinfelder et al. 1998). Recent data on *Potamocorbula amurensis*, an invasive bivalve that now dominates the benthos of San Francisco Bay, suggest that this organism is unusually efficient at concentrating Se from phytoplankton because of their high AEs and low efflux rates (Schlekat et al. 2000). Thus, predators of these bivalves, such as sturgeon or diving ducks, may be particularly susceptible to changes in Se loadings to the Bay. Moreover, the striking shift in organic carbon flux from the plankton to the benthos that has resulted from the invasion of San Francisco Bay by *P. amurensis* may have the ancillary effect of increasing the efficiency of Se transport up the food web. San Francisco Bay may be a good example of an ecosystem that has undergone an ecologically mediated change in susceptibility to contaminant effects.

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