Environ. Sci. Technol. **2004**, *38,* 4519−4526

Food Web Pathway Determines How Selenium Affects Aquatic Ecosystems: A San Francisco Bay Case Study

A. ROBIN STEWART,*,† SAMUEL N. LUOMA,† CHRISTIAN E. SCHLEKAT,‡ MARTINA A. DOBLIN,§ AND KATHRYN A. HIEB||

U.S. Geological Survey, 345 Middlefield Road, Menlo Park, California 94025, Nickel Producers Environmental Research Association, 2605 Meridian Parkway, Suite 200, Durham, North Carolina 27713, Department of Ocean, Earth and Atmospheric Sciences, Old Dominion University, Norfolk, Virginia 23529, and California Department of Fish and Game, Central Valley Bay-Delta Branch, 4001 North Wilson Way, Stockton, California 95205

Chemical contaminants disrupt ecosystems, but specific effects may be under-appreciated when poorly known processes such as uptake mechanisms, uptake via diet, food preferences, and food web dynamics are influential. Here we show that a combination of food web structure and the physiology of trace element accumulation explain why some species in San Francisco Bay are threatened by a relatively low level of selenium contamination and some are not. Bivalves and crustacean zooplankton form the base of two dominant food webs in estuaries. The dominant bivalve *Potamocorbula amurensis* has a 10-fold slower rate constant of loss for selenium than do common crustaceans such as copepods and the mysid *Neomysis mercedis* (rate constant of loss, k_e = 0.025, 0.155, and 0.25 d^{-1}, respectively). The result is much higher selenium concentrations in the bivalve than in the crustaceans. Stable isotope analyses show that this difference is propagated up the respective food webs in San Francisco Bay. Several predators of bivalves have tissue concentrations of selenium that exceed thresholds thought to be associated with teratogenesis or reproductive failure (liver Se >15 $\mu g\ g^{-1}$ dry weight). Deformities typical of selenium-induced teratogenesis were observed in one of these species. Concentrations of selenium in tissues of predators of zooplankton are less than the thresholds. Basic physiological and ecological processes can drive wide differences in exposure and effects among species, but such processes are rarely considered in traditional evaluations of contaminant impacts.

Introduction

Large investments have been made in controlling chemical contamination of aquatic environments; however, identification of the ecological significance of contaminants in complex environmental settings remains problematic (*1*). One example of a significant effect on wildlife was the discovery of selenium (Se) poisoning at Kesterson Reservoir, CA, in 1983 (*2*, *3*). Selenium, concentrated in irrigation drainage from the Western San Joaquin Valley (in the Central Valley of California; 300 $\mu g\ L^{-1}$; 3.8 mM), was transported into the Kesterson National Wildlife Refuge where it was accumulated by nesting birds, resulting in a significant deformity rate in bird hatchlings (64% nests affected for eared grebe, *Podiceps nigricollis*, and American coot, *Fulica americana*; *4*).

Concentrations of Se in solution and sediments were much reduced downstream from the reservoir, in San Francisco Bay (<1 $\mu g\ L^{-1}$ in water) (*5*, *6*). Nevertheless, concentrations in some predatory fish (e.g., white sturgeon, *Acipenser transmontanus*) and some predatory birds (e.g., scoter, *Melanitta perspicillata*) were high (>10 $\mu g\ g^{-1}$ dry weight) (*7*, *8*), while concentrations in other important predatory and prey species (striped bass, *Morone saxatilis*) were much lower. In this study we ask the question: Why did concentrations of Se differ so widely among predators in the Bay, and do those differences still occur? Does food web biomagnification of Se occur, and if so, why is it reflected differently in different predator species? Can stable isotopes be used help characterize different Bay food webs and help understand Se distributions? On the basis of bioaccumulation and stable isotope results, can we suggest what animals might potentially be most threatened by Se, and is there any evidence that effects are occurring in those specific species? Traditional evaluations of the implications of contamination, which include toxicological testing, geochemical speciation, or changes in community structure (*5*), do not address such questions. They may explain acute toxicity, cycling, sources, and bioavailability but not why species differ in their responses. So the issue of which species are most vulnerable to contamination remains poorly known.

Recent work shows that diet can be critical in determining contaminant exposures of animals. Where there is a strong dietary link in contaminant exposures, exposures of top predators can be explained by food web relationships (*9*). Diet dominates Se uptake (*10*, *11*), but recent attempts to relate Se distributions to food webs have met with limited success (*12*). We also ask whether that lack of success stems from different processes affecting contaminant uptake by invertebrates at the lower trophic levels.

Experimental Section

Field Sampling. To limit the confounding influences of temporal and spatial variability in comparisons among species, sampling was constrained to a specific geographical area and season. Sampling for Se concentrations in invertebrates and fish was constrained to Suisun Bay and closely contiguous habitat in the northern reach of San Francisco Bay (Figure 1). We assume a homogeneous distribution of Se throughout the study region. Suisun Bay is near the head of the estuary, seaward from the confluence of the Sacramento−San Joaquin River system. It is a major part of the migration corridor and feeding ground for anadromous fish (e.g., Chinook salmon, *Oncorhynchus tshawytscha*; white sturgeon; and striped bass) and seasonally is a nursery area for fish that spawn either in freshwater (e.g., Sacramento splittail, *Pogonichthys macrolepidotus*; striped bass) or the ocean (e.g., Dungeness crab, *Cancer magister*; starry flounder, *Platichthys stellatus*). For this specific study, only samples collected in fall and early winter of 1999/2000 were used.

* Corresponding author phone: (650)329-4550; fax: (650)329-5590; e-mail: arstewar@usgs.gov.
† U.S. Geological Survey.
‡ Nickel Producers Environmental Research Association.
§ Old Dominion University.
|| California Department of Fish and Game.

10.1021/es0499647 CCC: $27.50
Published on Web 08/03/2004

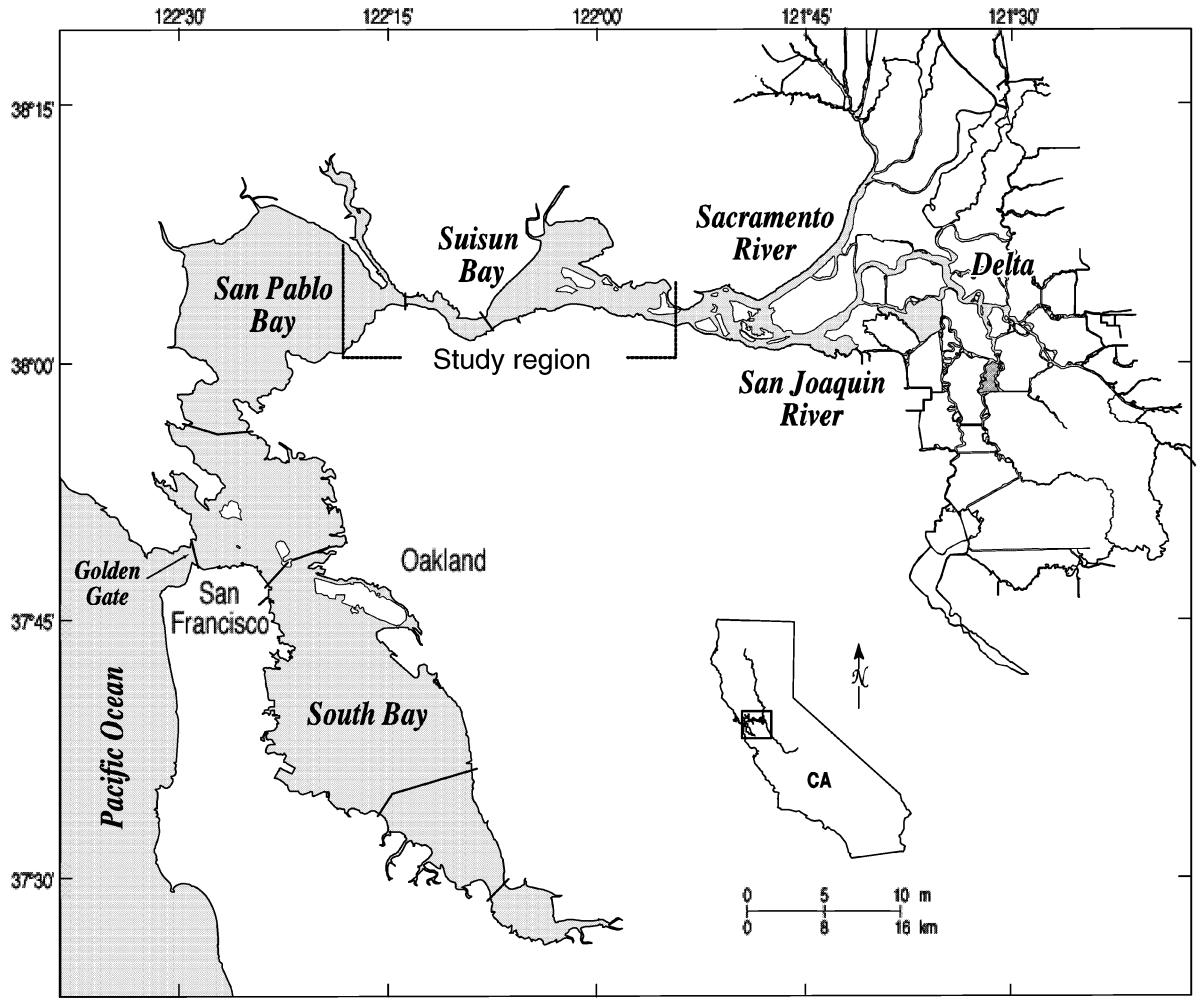

FIGURE 1. Map of San Francisco Bay and Delta showing study region where samples were collected.

This exploited the period when juvenile fishes and crab (collected November and December 1999) were resident in northern San Francisco Bay and when migratory species, such as white sturgeon (collected January 2000) and striped bass (collected December 1999), may have spent several weeks or months feeding in the region. This is also when Se concentrations in key invertebrate species are at their peak (*13*, *14*). Zooplankton were collected in November, the clam *Potamocorbula amurensis* was collected monthly from August through November, and the remaining invertebrates were collected in October 1999. Fish and Dungeness crab were collected by otter trawls, by beach and purse seines, and from anglers. Large fish including sturgeon, striped bass, starry flounder, and leopard shark (*Triakis semifasciata*) were filleted within 6 h of collection to remove muscle fillets (skin removed) and livers, which were then frozen in plastic Ziploc bags. Smaller fish (juvenile striped bass; yellowfin goby, *Acanthogobius flavimanus*; and Sacramento splittail) and Dungeness crab were frozen whole in plastic Ziploc bags until they were dissected in the laboratory to remove muscle and liver or hepatopancreas (Dungeness crab). Zooplankton were collected using vertical tows of a 75-μm mesh net at three locations within the study region and one location below San Pablo Bay closer to the marine end of the estuary. In the field, bulk zooplankton samples were further filtered through a 2000-μm mesh screen, resulting in a final plankton size range of 75−2000 μm, and transferred to acid-clean polyethylene vials. Samples collected at the same time for another study indicate that the zooplankton species were similar among sites and were primarily copepods (*Acartia* spp., Oithonidae, *Paracalanus* spp., *Psuedodiaptomus* spp., *Tortanus dextrilobatus,* and copepod nauplii) with the predator Oithonidae contributing 75% of the samples' biovolume (*13*). Amphipods, isopods and the shrimp, *Crangon franciscorum*, were collected by benthic sled and zooplankton net, pooled by sampling location (n = 3−4 composites) and stored in plastic Ziploc bags. In the laboratory, amphipods were further sorted by species (*Ampelisca abdita* and *Corophium* spp.*)* and frozen separately in acid-washed polyethylene vials. *Corophium alienense* and *Corophium stimpsoni* were combined into a single composite (i.e. *Corophium* spp.) per location due to insufficient sample mass for individual species analyses. Isopods, *Gnorimosphaeroma oregonensis* and *Synidotea laevidorsalis*, were also combined into a single composite at each location. *P. amursensis* were collected by benthic grab during routine monthly cruises; depurated for 48 h (*15*); and their soft tissues were removed from shells, pooled by sampling location (n = 6), and frozen in acid-washed polyethylene vials. Most other invertebrate samples were analyzed whole without depurating their gut contents. Brown and Luoma (*15*) showed that the influence of gut content on tissue burdens in invertebrates is determined by metal concentrations in the food (of which particulate material is a good indicator for most species). Ingested food such as sediments or suspended particulates is expected to have little influence on the overall Se body burden of the invertebrates since Se concentrations in these phases are relatively low as compared to those in the organisms (Suisun Bay sediment, 0.24 μg g^{-1} dry weight, n = 13, S. Meseck, NOAA, personal communication; Suisun Bay suspended

particulates in the fall of 1997−1999, 0.648 μg g^{-1} dry weight, n = 21, Doblin, unpublished data).

Stable Isotopes. To determine whether differences in Se uptake in invertebrates could explain variable concentrations in their predators we identified predator−prey relationships within San Francisco Bay using stable isotopes and available dietary information. Stable nitrogen isotope ratios (δ^{15}N) provide a spatially and temporally integrated measure of trophic relationships in a food web (i.e., primary producers → invertebrates → fish) because δ^{15}N becomes enriched by 2.5−5‰ between prey and predator (*16*). Stable carbon isotope ratios (δ^{13}C) show little or no enrichment (<1‰) with each trophic level but can identify contributions of different foods if foods have distinct isotopic signatures (*17*). In estuaries, algal carbon isotopic signatures are influenced by the δ^{13}C values of dissolved inorganic carbon (DIC); δ^{13}C is enriched with increasing salinities (*18*). Thus, as DIC is incorporated into the base of the food web, the resulting δ^{13}C in consumers varies according to their predominant foraging location along the salinity gradient.

Food chain length was calculated for clam and crustacean food webs by subtracting the lowest δ^{15}N value from the highest δ^{15}N value in each food web. Baseline nitrogen signatures in bivalves and zooplankton did not vary along the estuarine gradient, which allowed for a legitimate comparison of food chain length among food webs (*19*, *20*).

Individual fish and crab muscle, individual whole shrimp, soft tissues of clams, and pooled whole zooplankton, amphipods, and isopods were analyzed for δ^{13}C and δ^{15}N at the Stable Isotope Facility, University of California, Davis, CA, using a Europa Scientific Hydra 20/20 continuous flow isotope ratio mass spectrometer and Europa ANCA-SL elemental analyzer to convert organic C and N into CO_2 and N_2 gas. Results are presented as deviations from standards, expressed as δ^{13}C and δ^{15}N:

$$\delta X = [R_{\text{sample}}/R_{\text{standard}} - 1] \times 10^3 \qquad (1)$$

where X is ^{13}C or ^{15}N and R is ^{13}C/^{12}C or ^{15}N/^{14}N. The standard for C is Peedee Belemnite, and for N it is atmospheric diatomic nitrogen. Instrument precision was 0.1‰ for carbon and 0.3‰ for nitrogen based on replicate analyses of standard reference materials.

Selenium Analyses. Analyses were conducted on individual fish livers and *C. magister* hepatopancreas except for yellowfin goby and Sacramento splittail whose livers, due to insufficient sample mass for Se analysis, were pooled by individuals with similar muscle isotopic δ^{13}C and δ^{15}N values. Invertebrates were analyzed whole (see above for stable isotopes) from composites of 20−>100 individuals. Several different composites were analyzed for every species (number of replicates for each species is shown in figures). Samples of large mass (fish, crab, clams, and shrimp) were analyzed using oxidative digest and selective hydride generation atomic absorption spectroscopy (AAS) (*14*). Fish liver, crab hepatopancreas, clam soft-tissues, and whole shrimp samples (stored at −30 °C for <6 months) were dried at 40 °C, weighed and subsequently digested in concentrated nitric and perchloric acids at 200 °C, reconstituted in hydrochloric acid, and then stored until analysis. Quality control was maintained by frequent analysis of blanks, analysis of National Institute of Standards and Technology (NIST) standard reference materials with each analytical run, and internal comparisons with prepared quality control standards. Samples of small mass (zooplankton, amphipod, and isopod) were also determined using oxidative digest and selective hydride generation atomic absorption spectroscopy (AAS), but with a three-step nitric−perchloric acid reflux procedure (*21*). After evaporation of the nitric acid, the residue was redissolved in 4 M HCl and stored until final Se analysis. To determine Se concentrations, 1−2-mL aliquots of digest solution were diluted to 40 mL with distilled water in a 400-mL glass beaker to which Teflon boiling stones, 0.5 mL of 2% (w/v) persulfate solution, and 22 mL of concentrated HCl were added. The beaker was covered with a watch glass, and the solution was brought to a boil for 30 min, with the heat being reduced to the minimum capable of sustaining boiling. After cooling overnight, the samples were analyzed using hydride generation. The standard additions method of calibration was used to ensure accuracy, and all determinations were made in triplicate to establish precision. In addition to the standard addition method, accuracy was verified using the digestion and determination of Se in NIST Oyster Tissue with each group of 10 samples. All sample weights were corrected for salt content by measuring Na concentrations using flame AAS. Selenium concentrations are expressed on a dry weight basis.

Bioaccumulation Parameters. A dynamic multi-pathway bioaccumulation model (DYMBAM) approach to bioaccumulation was used (*22*, *23*) to help characterize processes influencing uptake of Se and to predict steady-state tissue concentrations (C_{ss}) in the lower trophic level organisms, from both a benthic bivalve-based food chain and a pelagic crustacean-based food chain. The model predicts metal accumulation from waterborne and dietborne uptake routes (*10*, *24*), and is expressed as

$$C_{ss} = (k_u C_W)/(k_e) + (\text{AE} \times \text{IR} \times C_F)/(k_e) \qquad (2)$$

where k_u is the dissolved metal uptake rate constant (L g^{-1}d^{-1}), C_W is the dissolved metal concentration (μg L^{-1}), AE is the assimilation efficiency (%), IR is the ingestion rate (g g^{-1}d^{-1}), C_F is the metal concentration in food (e.g., phytoplankton, suspended particulate matter, sediment) (μg g^{-1}), and k_e is the efflux rate from waterborne and dietborne metal, respectively (d^{-1}). Data and model parameters are available for a pelagic-based food web of phytoplankton (diatoms) to herbivorous zooplankton to carnivorous zooplankton (*25*, *26*) and for a benthic-based food web of phytoplankton to bivalves (*27*−*29*). The DYMBAM model was used to estimate steady-state Se concentrations (C_{ss}) in the mysid *Neomysis mercedis*, which is endemic to SFB, and in the bivalve *P. amurensis*, as these steady-state concentrations represent dietary exposure levels for the pelagic and benthic food chains.

Uptake kinetics from solution and rate constants of loss (Se k_u and k_e) for the pelagic-based food web were obtained from experiments with herbivorous zooplankton (mixed species of copepods; *13*) and carnivorous zooplankton (*N. mercedis*) (*29*). All experimental animals were collected from San Francisco Bay. AEs for copepods feeding on diatoms (*Phaeodactylum tricornutum*) and for mysids feeding on copepods were also reported in this study. For *P. amurensis*, Luoma et al. (*10*) originally showed that uptake from solution was irrelevant. So only Se AEs, which range from from 45% to 80%, were used to account for the Se uptake and differences with which this species assimilates Se from different phytoplankton species (*27*, *28*). Sources of other physiological and uptake kinetic data are indicated in Table 1.

Average dissolved Se concentrations for San Francisco Bay are approximately 0.25 μg L^{-1} (*5*), and the range of particulate Se concentrations in San Francisco Bay is 0.5−1.5 μg g^{-1} (Doblin, unpublished data). These values were used as water and food concentrations (i.e., C_W and C_F, respectively) for the DYMBAM model.

Data Analysis. The software SYSTAT 10 was used for all statistical analyses. We used ANOVA and Tukey HSD for unequal n to test for differences in Se concentrations among invertebrate species. To test the significance of the relation-

TABLE 1. Bioaccumulation Model for the Crustacean *Neomysis mercedis* and the Bivalve *Potamocorbula amurensis*[a]

Food chain	Species	k_u (L g^{-1} d^{-1})	IR (g g^{-1} d^{-1})	AE (%)	k_e (d^{-1})	C_{ss} (μg g^{-1})
Bivalve	*P. amurensis*	0.003[b]	0.25[b]	45−80[c,d]	0.025[e]	2.1−12
Mysid	Copepods	0.024[f]	0.42[f]	50−53[e]	0.155[e]	0.7−2.2
	N. mercedis	0.027[e]	0.45[g]	73[e]	0.25[e]	0.9−2.7

[a] Mysid model was from diatoms to copepods to mysids, and bivalve model was from diatoms to bivalves. A single dissolved concentration (0.3 μg L^{-1}) and a range of particulate concentrations (0.5−1.5 μg g^{-1}) were used to predict steady-state tissue concentrations ($C_{ss} = ((k_u C_W)/k_e) + ((AE \times IR \times C_F)/k_e)$, where k_u is the dissolved metal uptake rate constant (L g^{-1} d^{-1}), C_W is the dissolved metal concentration (μg L^{-1}), AE is the assimilation efficiency (%), IR is the ingestion rate (g g^{-1} d^{-1}), C_F is the metal concentration in food (μg g^{-1}), and k_e is the efflux rate (d^{-1}). [b] USGS, unpublished data. [c] Ref *27*. [d] Ref *28*. [e] Ref *29*. [f] Ref *25*. [g] Ref *26*.

ships between Se concentrations and trophic level ($\delta^{15}N$) we used linear regression.

Toxicity Thresholds. "Toxicity thresholds" are used in this paper to provide some perspective on the Se concentrations in tissues of both invertebrates and fish. The primary route of Se exposure to fish and invertebrates in nature is diet (*10*, *30*). In field studies, predators (fish) are the most sensitive species in the food web (e.g., ref *31*). The thresholds considered here are only from studies with predators and those that address concentrations that cause or coincide with teratogenesis or reproductive failure (the most sensitive end points). Thresholds for determining toxicity to predators are of two types. Threshold concentrations in food that cause adverse effects in predators and threshold concentrations in tissues of the predators themselves that coincide with the onset of effects. The predators for which the thresholds were derived were not the specific species we studied in San Francisco Bay.

In reviewing existing literature, Lemly (*30*) showed that concentrations of Se greater than 3 μg g^{-1} in the diet of fish result in deposition of elevated Se concentrations in developing eggs, particularly the yolk. Dietary Se concentrations within the range of 5−20 μg g^{-1} load eggs with Se beyond their teratogenic threshold. In the field, extinctions of numerous species of fish were observed in Belews Lake, in association with Se concentrations in invertebrates in the concentration range of 20−80 μg g^{-1} dry weight (*31*). We display a threshold value of 10 μg g^{-1} dry weight as representative of the field/laboratory range.

Lemly (*30*) also listed the proportion of deformities that were observed at different concentrations in fish tissues. In a variety of studies, the appearance of teratogenesis began at 5−10 μg g^{-1} dry weight whole tissue. High proportions of young were deformed above 20 μg g^{-1} dry weight whole tissue. Teratogenesis and reproductive failure consistently began to appear at tissue concentrations in excess of 15 μg g^{-1} dry weight. We chose to display 15 μg g^{-1} dry weight as representative of the threshold concentration in liver of fish. For both food and tissue thresholds, we recognize that the database is limited, the threshold may differ among species, and experts differ somewhat about the exact value representing a threshold (*32*).

Results and Discussion

Selenium concentrations ranged from low to potentially toxic in both invertebrates and fish (Figure 2A,B). Concentrations in lower trophic level crustaceans such as amphipods (*Ampelisca abdita*) ranged from 1 to 3 μg g^{-1} (dry weight) and were as high as 6 μg g^{-1} in zooplankton (although some of these were predaceous; *13*). In contrast, concentrations of Se in the filter-feeding bivalve, *P. amurensis*, were significantly higher than all the crustaceans at 5−20 μg g^{-1} (ANOVA, $P < 0.0001$) (Figure 2A). Suspended particulate Se concentrations in northern San Francisco Bay are relatively low, typically between 0.5 and 1.5 μg g^{-1} (Doblin, unpublished data). Thus, compared to suspended particulate material, Se is significantly biomagnified in *P. amurensis*, slightly biomagnified in zooplankton, and simply accumulated in other crustaceans.

Selenium uptake and elimination kinetics were examined to determine if these rates could explain the marked differences in concentrations seen in the field between clams and crustaceans. Both the bivalve *P. amurensis* and the mysid *N. mercedis* efficiently assimilated Se from their food (AEs > 50%) and accumulated dissolved Se slowly (Table 1). Neither AE nor uptake from solution differed greatly between the two species; so another explanation is needed for the differences in bioaccumulated Se seen in nature. The parameter that differed the most between bivalves and crustaceans was the elimination rate (k_e), which was 10 times lower for *P. amurensis* (0.025 d^{-1}) than for *N. mercedis* (0.25 d^{-1}) (Table 1). Results of the DYMBAM model, using Se concentrations in water and particulate material from Suisun Bay, showed that slower rates of elimination in bivalves resulted in higher steady-state concentrations for bivalves (maximum $C_{SS} = 12$ μg g^{-1}) than mysids (maximum $C_{SS} = 2.1$ μg g^{-1}) (Table 1). The DYMBAM forecasts also agreed reasonably closely with concentrations observed in these species in the Bay.

One physiological mechanism that might explain differences in Se loss between bivalves and crustaceans is the greater tendency of marine bivalves to re-absorb amino acids, or perhaps small proteins, that they lose as a result of catabolism. Selenium primarily occurs associated with proteins in the tissues of organisms and presumably is lost in that form. Wright and Manahan (*33*) and Manahan (*34*) showed direct absorption of dissolved organic material (DOM) or amino acids can occur across body surfaces of many soft-bodied marine invertebrate phyla, including bivalves. But the exception is marine arthropods, for which re-absorption is not efficient. A perhaps related phylogenetic distinction was observed by Schlekat et al. (*28*), who reported strong relationships between AEs and the proportion of Se in algal cell cytoplasm (in the form of dissolved organic selenides) for bivalves (including *P. amurensis*) but not for the amphipod *Leptocheirus plumulosus*.

Selenium concentrations were also highly variable among upper trophic level consumers including crab and fish. Mean liver Se concentrations ranged from 3.6 μg g^{-1} in yellowfin goby to 24 μg g^{-1} in white sturgeon (Figure 2B). Patterns in accumulation among the different species were not related to size or age. For example, larger, older Sacramento splittail (length 18 cm; age 1−2 yr; *35*) like white sturgeon (length 135−171 cm; age 14−20 yr; *36*) accumulated Se beyond the toxicity threshold to levels that have been correlated with adverse reproductive effects; but adult striped bass (length 49−94 cm; 3−10 yr; *37*) had much lower concentrations. Selenium is typically not detoxified in animal tissues by conjugation with metal-specific proteins or association with nontoxic inclusions. So mechanisms that semi-permanently sequester other metals and lead to progressive accumulation with size or age are not known for Se.

To determine whether differences in Se concentrations among fish could be explained by food-related variables, we examined feeding relationships among biota from San Francisco Bay. Stable isotope results were consistent with known dietary habits and gut-contents studies of the species collected and together were used to identify two crustacean-based and one clam-based food web along the salinity gradient (Figure 3). In Figure 3, ellipses enclose animals thought to be in similar food webs from knowledge of their

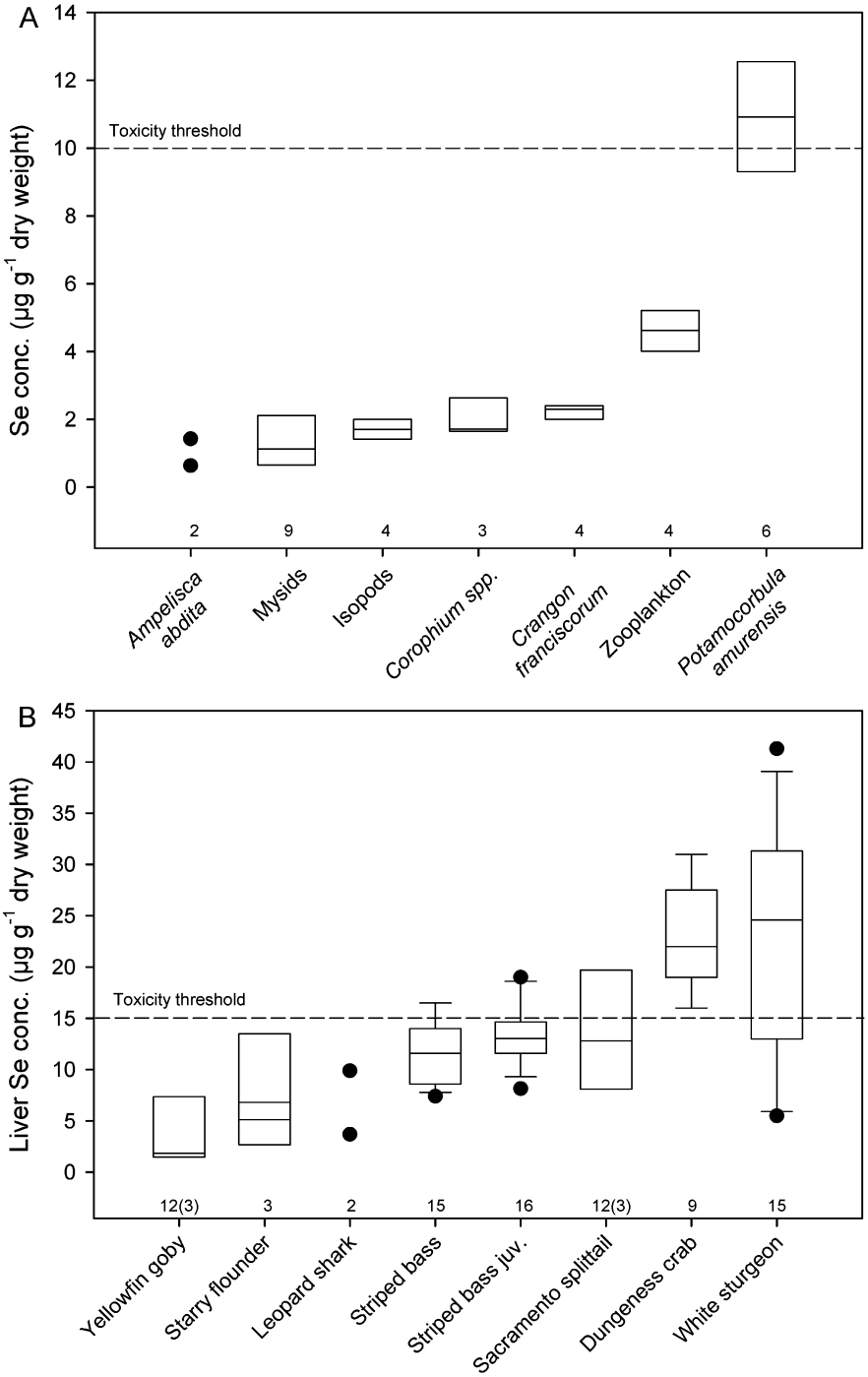

FIGURE 2. Selenium concentrations (μg g^{-1}, dry weight) in invertebrate and predator species named on the ordinate. Animals were all from North San Francisco Bay and collected in the fall and winter of 1999/2000. (A) Invertebrates: dashed line represents the toxicity threshold for predator food defined in the text. Mysids were *Neomysis mercedis* from Purkerson et al. (*13*). Isopods were a mixture of *Gnorimosphaeroma oregonensis* and *Synidotea laevidorsalis*. *Corophium* spp. were a mixture of *Corophium alienense* and *Corophium stimpsoni*. Zooplankton were mixed species ranging in size from 75 to 2000 μm (*13*). (B) Predators: dashed line represents the threshold of toxicity for selenium in liver of predators, as explained in text. Yellowfin goby, *Acanthogobius flavimanus*; starry flounder, *Platichthys stellatus*; leopard shark, *Triakis semifasciata*; striped bass, *Morone saxatilis*; Sacramento splittail, *Pogonichthys macrolepidotus*; Dungeness crab, *Cancer magister*; white sturgeon, *Acipenser transmontanus*. Box plots are defined as follows: The boundary of the box indicates the 25th and 75th percentile; a line within the box marks the median; whiskers above and below the box indicate the 90th and 10th percentiles; outlying points and data points for species with samples sizes of $n < 3$ are also shown. Number of individuals or composites (shown in brackets for yellowfin goby and Sacramento splittail) analyzed for selenium are shown.

feeding habits, showing those relationships within the context of the δ^{13}C and δ^{15}N values. Adult striped bass, leopard shark and starry flounder had enriched δ^{13}C values (−17 to −19‰) relative to other fish species, indicating that they were foraging toward the marine end of the estuary. Their δ^{13}C values were similar to crustacean isopods, amphipods, and zooplankton (−20‰), their potential prey. Indeed, starry flounder are known to feed on amphipods (*38*), which is supported in the isotope data by a 4‰ (~1 trophic level) enrichment of their mean δ^{15}N values. Adult striped bass typically feed on zooplanktivorous forage fish (*38*), and their δ^{15}N values were enriched by 8.6‰ (~2 trophic levels) as compared to

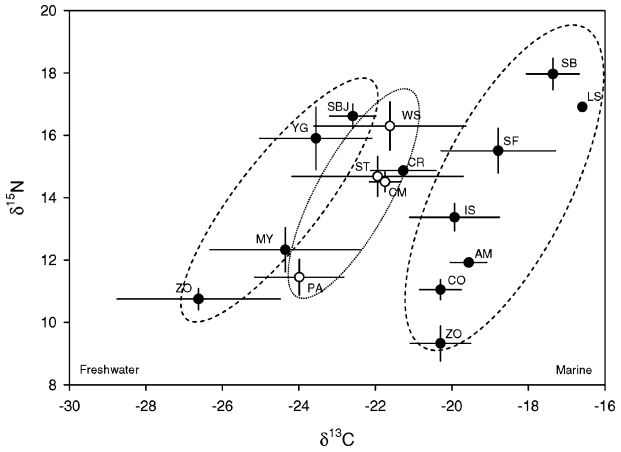

FIGURE 3. Stable isotope plot showing feeding relationships among fish and invertebrates in North San Francisco Bay in the fall and winter of 1999/2000. Values are means (±SD). Clam-based (open circles and short-dash ellipse) and crustacean-based (closed circles and long-dash ellipse) food webs are operationally identified using stable isotopes and dietary information. Due to the extreme differences in isotopic composition among samples zooplankton were separated into freshwater and marine samples. SB, *Morone saxatilus* (striped bass); SBJ, juvenile *M. saxatilus*; LS, *Triakis semifasciata* (leopard shark); SF, *Platichthys stellatus* (starry flounder); YG, *Acanthogobius flavimanus* (yellowfin goby); CR, *Crangon franciscorum* (shrimp); IS, isopod (*Gnorimosphaeroma oregonensis* and *Synidotea laevidorsalis*); AM, *Ampelisca abdita* (amphipod); CO, *Corophium alienense* and *Corophium stimpsoni*; (amphipod) MY, *Neomysis mercedis* (mysid); ZO, zooplankton (mixed species ranging in size from 75 to 2000 μm; *13*); WS, *Acipenser transmontanus* (white sturgeon); ST, *Pogonichthys macrolepidotus* (splittail); CM, *Cancer magister* (crab); PA, *Potamocorbula amurensis* (clam).

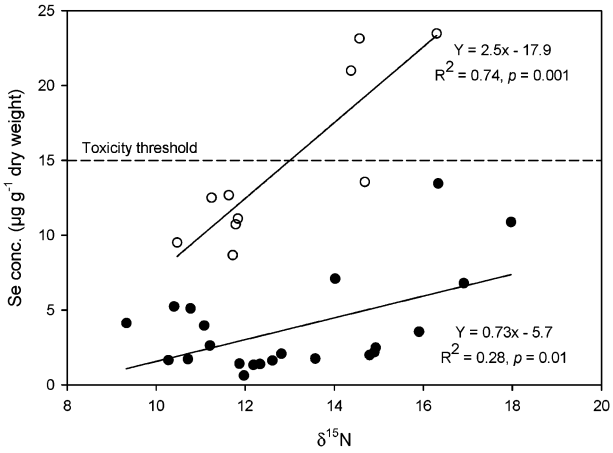

FIGURE 4. Selenium concentrations in organisms in the clam-based food web (open circles) compared to the crustacean-based food web (closed circles) in San Francisco Bay. Values are based on individual composites of invertebrate samples and mean selenium concentrations in predators shown in Figure 2. The food webs were those operationally identified within ellipses in Figure 3, by diet and stable isotope analysis. Dashed line represents the threshold of toxicity for selenium in liver of predators, as explained in text.

zooplankton. Carbon and nitrogen isotope ratios showed that juvenile striped bass, yellowfin goby, white sturgeon, Sacramento splittail, Dungeness crab, and *C. franciscorum* resided in fresher waters and were predators (Figure 3). Dietary studies show that these predators feed differently. For example, mysids (*N. mercedis*) are the primary food of juvenile striped bass and zooplankton are the prey of the mysids (*38*, *39*), which is consistent with a trophic enrichment of δ^{15}N values from zooplankton (10.75‰) to mysid (12.33‰) to juvenile striped bass (16.62‰). Since its introduction in 1986 (*40*), the clam *P. amurensis* has been a dominant food item in the digestive tracts of benthivorous sturgeon and older splittail (*39*). Isotopic nitrogen values for these fish are 4.85‰ and 3.24‰ more enriched than *P. amurensis* as would be expected if the clams contribute to the diet of these fish. The higher δ^{15}N values for sturgeon than splittail also indicate that sturgeon may also eat higher trophic level biota. Although no diet data exist for Dungeness crab in San Francisco Bay, similar isotopic values to those of splittail and studies of Dungeness crab in other estuaries (*41*, *42*) indicate that clams such as *P. amurensis* would be expected to be an important food for this species. Small crabs (range 15−60 mm) in Grays Harbor, WA, similar to those sampled in this study (mean width 50 mm) were found to consume primarily bivalves (*41*). *C. franciscorium* are omnivorous and have been shown to feed primarily on amphipods, consistent with its enriched δ^{15}N (~3.42‰) relative to the amphipods, but may also consume some bivalves, polychaetes, and isopods (*43*).

Although carbon isotopes are known to undergo little fractionation among trophic levels (<1‰) (*44*), enrichment of carbon isotopic values from prey to predators in the fresher regions of the estuary averaged 2‰ and 2.4‰ per trophic level for the juvenile striped bass and white sturgeon food webs, respectively. The larger enrichment values for these food webs may reflect differences in time averaging of isotopic signatures among trophic levels (*45*). There are large seasonal fluctuations in salinity in the northern portion of the estuary that co-vary with δ^{13}C values of DIC (0−25 psu and −9.1 to −2.9 δ^{13}C DIC; *18*) with the greatest variation at the lower salinities. As salinities shift throughout the year primary consumers respond quickly to shifts in δ^{13}C of the estuarine phytoplankton due to their small body size and rapid tissue-turnover rates (Stewart, unpublished data). In contrast, fish, shrimp, and crab spend part of their time foraging at higher salinities, which combined with their longer tissue turnover rates (*46*) would result in more enriched (marine) isotopic signatures in their tissues than their invertebrate prey. Differences in time averaging of isotopic signatures among trophic levels have been reported for other dynamic ecosystems (*45*) and typically do not lend themselves to two-source mixing models that are commonly used to determine the relative importance of food sources (e.g., benthic vs pelagic carbon).

In Figure 4, the food webs operationally identified in Figure 3 from both dietary and isotopic (δ^{15}N and δ^{13}C) information were labeled as clam-based or crustacean-based food webs and plotted against Se concentrations in animal tissues. The crustacean and clam food webs both appeared to biomagnify Se with trophic position, as shown by a significant relationship between Se and δ^{15}N (clam: $R^2 = 0.74$, $p = 0.001$; crustacean: $R^2 = 0.28$, $p = 0.01$) (Figure 4). But the accumulation of Se versus δ^{15}N in the two food webs showed different degrees of biomagnification. Selenium accumulation with trophic level was significantly greater through the clam-based food web relative to the crustacean-based food web (ANCOVA Se × δ^{15}N; $F = 8.8$, $p = 0.0062$). The best-fit regressions for the two food webs suggested that Se concentrations were typically about 5-fold different between clam- and crustacean-based food webs at the highest trophic levels observed in the Bay (Figure 4). Higher Se concentrations at the base of the clam food web and greater rates of Se trophic transfer led to Se concentrations in clam predators that exceeded the toxicity threshold for tissues (*47*), while those in crustacean predators did not.

Reinfelder et al. (*48*) and Wang (*49*) both suggested, on a theoretical basis, that differences in efflux rates could be one factor that determines the degree to which an element biomagnifies up food webs. But, to our knowledge there are

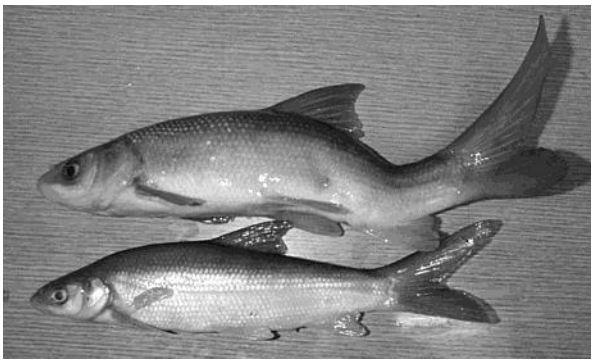

FIGURE 5. Sacramento splittail collected from North San Francisco Bay, CA, in 1999 displaying lordosis, a selenium-induced teratogenic deformity. Photo taken by Fred Feyrer, California Department of Water Resources.

no other studies that demonstrate, in the field, specific differences in Se uptake among primary consumers propagating to differences in contaminant accumulation at the top of the food web. In San Francisco Bay, processes that control Se uptake at the base of the food web appear to be the dominant factor controlling which species among top predators are exposed to the highest concentrations of this potential toxin.

The clam food web was also of the same length or shorter than either of the crustacean food webs (clam food web $\Delta\delta^{15}N = 4.9$; crustacean food web $\Delta\delta^{15}N = 7.6$). Therefore, food web length was not the most important factor determining Se concentrations in top predators. These results are contrary to those of other biomagnifying contaminants such as polychlorinated biphenyls, DDT, or mercury, which identify food chain length or carbon source as being a critical factor controlling concentrations in top predators (*50*, *51*). Additional anecdotal evidence highlights the relative importance of food web length and food web base concentrations in determining Se exposures. One exceptional white sturgeon (of 37 collected over 2 yr) appeared to be piscivorous, based upon $\delta^{15}N$ (18.75‰ vs a group mean = 15.65). That individual had the lowest liver Se level (5.5 $\mu g\ g^{-1}$, group mean = 22 $\mu g\ g^{-1}$). Conversely, this same sturgeon had the highest muscle mercury level (3.68 $\mu g\ g^{-1}$, dry weight; group mean = 1.12 $\mu g\ g^{-1}$) relative to other sturgeon. High Se concentrations were also observed in Dungeness crab (22 $\mu g\ g^{-1}$). Loss rates of Se are not known for this species, but it is a crustacean. It also preys upon clams; and, like other species in that food web, the clams are likely the source of the elevated Se. So food chain length can play a role in magnifying concentrations from one trophic level to the next (Se concentrations increase from clams to their predators), but that process only enhances the most significant increase resulting from enhanced uptake at the base of the food web.

A principal effect of Se is teratogenicity. Deformities occur in developing embryos when Se replaces sulfur in sulfur-rich hard tissues (*52*). Recent field surveys identified Sacramento splittail from Suisun Bay (where Se concentrations are highest) that have deformities typical of Se exposure (Figure 5). This suggests a toxicologic threat in at least some individuals of an important native species that has been listed under the Endangered Species Act (50 CFR Part 17).

Variable exposures among species complicate interpreting the influences of contaminants in nature. For this reason traditional approaches to understanding the environmental threat of contaminants may not be appropriate for Se. Biomagnification in San Francisco Bay makes upper trophic levels most vulnerable to Se effects. But unlike other contaminants that biomagnify, differences in the kinetics of uptake and loss at the first trophic step (clams and crustaceans) and propagation of those differences up trophic pathways cause some predators to be more exposed than others to Se. Presumably, this can influence what species might be most likely to disappear from a moderately contaminated environment. Similar principles may apply to other contaminants where diet is an important route of exposure (*49*). Combining ecological and environmental toxicological approaches, at the ecosystem level, with mechanistic laboratory experimentation, may help understand if effects of chemicals such as Se are going undetected in natural populations from such environments.

Acknowledgments

We thank D. W. Schindler, N. S. Fisher, G. A. Cutter, M.-N. Croteau, and Theresa Presser for their helpful comments on the manuscript and the USGS Menlo Park Metals Bioavailability Laboratory and crew of the R/V *Polaris* for assistance with sample collection. Research was supported by grant to S.N.L. and others from the CALFED Bay Delta Program (CALFED 98-2015000-00096), as well as from the NSF EGB Program (NSF Grant OCE-9707946) to G. A. Cutter.

Literature Cited

(1) Larison, J. R.; Likens, G. E.; Fitzpatrick, J. W.; Crock, J. G. *Nature* **2000**, *406*, 181−183.
(2) Presser, T. S. *Environ. Manage.* **1994**, *18*, 437−454.
(3) Presser, T. S.; Ohlendorf, H. M. *Environ. Manage.* **1987**, *11*, 805−821.
(4) Ohlendorf, H. M.; Hoffman, D. J.; Saiki, M. K.; Aldrich, T. W. *Sci. Total Environ.* **1986**, *52*, 49−63.
(5) Cutter, G. A. *Estuarine Coastal Shelf Sci.* **1989**, *28*, 13−34.
(6) Johns, C.; Luoma, S. N.; Elrod, V. *Estuarine Coastal Shelf Sci.* **1988**, *27*, 381−396.
(7) Lemly, A. D. In *Environmental Contaminants in Wildlife*; Beyer, W. N., Heinz, G. H., Redmon-Norwood, A. W., Eds.; CRC Press: Boca Raton, FL, 1996; Chapter 19, pp 427−446.
(8) Skorupa, J. P. In *Environmental Chemistry of Selenium*; Frankenberger, W. T., Jr., Engberg, R. A., Eds.; Marcel Dekker: New York, 1998; Chapter 18, pp 315−354.
(9) Kidd, K. A.; Schindler, D. W.; Muir, D. C. G.; Lockhart, W. L.; Hesslein, R. H. *Science* **1995**, *269*, 240−242.
(10) Luoma, S. N.; Johns, C.; Fisher, N. S.; Steinberg, N. A.; Oremland, R. S.; Reinfelder, J. R. *Environ. Sci. Technol.* **1992**, *26*, 485−491.
(11) Lemly, A. D. In *Environmental Chemistry of Selenium*; Frankenberger, W. T., Jr., Engberg, R. A. Eds.; Marcel Dekker: New York, 1998; Chapter 16, pp 281−296.
(12) Jarman, W. M.; Hobson, K. A.; Sydeman, W. J.; Bacon, C. E.; McLaren, E. B. *Environ. Sci. Technol.* **1996**, *30*, 654−660.
(13) Purkerson, D. G.; Doblin, M. A.; Bollens, S. M.; Luoma, S. N.; Cutter, G. A. *Estuaries* **2003**, *26*, 956−969.
(14) Linville, R. G.; Luoma, S. N.; Cutter, L.; Cutter, G. A. *Aquat. Toxicol.* **2002**, *57* (1−2), 51−64.
(15) Brown, C. L.; Luoma, S. N. *Mar. Ecol. Prog. Ser.* **1995**, *124*, 129−142.
(16) Peterson, B. J.; Fry, B. *Annu. Rev. Ecol. Syst.* **1987**, *18*, 293−320.
(17) France, R. L. *Limnol. Oceanogr.* **1995**, *40* (7), 1310−1313.
(18) Spiker, E. C.; Schemel, L. E. In *Distribution and Stable-Isotope Composition of Carbon in San Francisco Bay*; Conomos, T. J., Ed.; American Association of Advanced Science: 1979; pp 195−212.
(19) Cabana, G.; Rasmussen, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10844−10847.
(20) Vander Zanden, M. J.; Rasmussen, J. B. *Ecology* **1999**, *80* (4), 1395−1404.
(21) Cutter, G. A. *Anal. Chem.* **1985**, *57*, 2951−2955.
(22) Luoma, S. N.; Fisher, N. In *Ecological Risk Assessments of Contaminated Sediments*; Ingersoll, C. G., Dillon, T., Biddinger, G., Eds.; SETAC Press: Pensacola, FL, 1997; pp 211−239.
(23) Schlekat, C. E., Lee, B.-G.; Luoma, S. N. In *Coastal and Estuarine Risk Assessment*; Newman, M. C.; Roberts, M. H.; Hale, R. C. Eds.; CRC Press: Boca Raton, FL, 2001; pp 151−188.
(24) Wang, W. X.; Fisher, N. S. *Sci. Total Environ.* **1999**, *237−238*, 459−472.
(25) Wang, W.-X.; Fisher, N. S. *Limnol. Oceanogr.* **1998**, *43*, 273−283.
(26) Johnston, N. T.; Lazenby, D. C. *Can. J. Zool.* **1982**, *60*, 813−824.
(27) Schlekat, C. E.; Dowdle, P. R.; Lee, B.-G.; Luoma, S. N.; Oremland, R. S. *Environ. Sci. Technol.* **2000**, *34*, 4504−4510.

(28) Schlekat, C. E.; Lee, B.-G.; Luoma, S. N. *Mar. Ecol. Prog. Ser.* **2002**, *237*, 79−85.
(29) Schlekat, C. E.; Purkerson, D. G.; Luoma, S. N. *Environ. Toxicol. Chem.* (**2004**, in press).
(30) Lemly, A. D. *Ecotoxicol. Environ. Saf.* **1997**, *37*, 259−266.
(31) Lemly, A. D. *Ecotoxicol. Environ. Saf.* **1993**, *26*, 181−204.
(32) Adams, W. J.; Brix, K. V.; Cothern, K. A.; Tear, L. M.; Cardwell, R. D.; Fairbrother, A.; Toll, J. E. In *Environmental Toxicology and Risk Assessment*, 7th ed.; *ASTM STP 1333*; Little, E. E., DeLonay, A. J., Greenberg, B. M., Eds.; American Society for Testing and Materials: West Conshohocken, PA, 1998; pp 312−342.
(33) Wright, S. H.; Manahan, D. T. *Annu. Rev. Physiol.* **1989**, *51*, 585−600.
(34) Manahan, D. T. *Am. Zool.* **1990**, *30*, 147−160.
(35) Caywood, M. L. California State University, Sacramento, CA, 1974.
(36) Kohlhorst, D. W.; Millier, L. W.; Orsi, J. J. *Calif. Fish Game* **1980**, *66*, 83−95.
(37) Collins, B. W. *Calif. Fish Game*. **1982**, *68*, 146−159.
(38) Feyrer, F. California State University, Sacramento, CA, 1999.
(39) Feyrer, F.; Herbold, B.; Matern, S. A.; Moyle, P. B. *Environ. Biol. Fish.* **2003**, *67*, 277−288.
(40) Carlton, J. T.; Thompson, J. K.; Schemel, L. E.; Nichols, F. H. *Mar. Ecol. Prog. Ser.* **1990**, *66*, 81−94.
(41) Stevens, B. G.; Armstrong, D. A.; Cusimano, R. *Mar. Biol.* **1982**, *72*, 135−145.
(42) Skilleter, G. A. *Mar. Ecol. Prog. Ser.* **1994**, *109*, 29−42.
(43) Wahle, R. A. *J. Crust. Biol.* **1985**, *5*, 311−326.
(44) Vander Zanden, M. J.; Rasmussen, J. B. *Limnol. Oceanogr.* **2001**, *46*, 2061−2066.
(45) O'Reilly, C. M.; Hecky, R. E. *Limnol. Oceanogr.* **2002**, *47*, 306−309.
(46) Hesslein, R. H.; Hallard, K. A.; Ramlal, P. *Can. J. Fish. Aquat. Sci.* **1993**, *50*, 2071−2076.
(47) Lemly, A. D. *Selenium Assessment in Aquatic Ecosystems*; Springer-Verlag: New York, 2002; p 161.
(48) Reinfelder, J. R.; Fisher, N. S.; Luoma, S. N.; Nichols, J. W.; Wang, W.-X. *Sci. Total Environ.* **1998**, *219*, 117−135.
(49) Wang, W.-X. *Mar. Ecol. Prog. Ser.* **2002**, *243*, 295−309.
(50) Kidd, K. A.; Bootsma, H. A.; Hesslein, R. H.; Muir, D. C. G.; Hecky, R. E. *Environ. Sci. Technol.* **2001**, *35*, 14−20.
(51) Cabana, G.; Rasmussen, J. B. *Nature* **1994**, *372*, 255−257.
(52) Diplock, A. T.; Hoekstra, W. G. *CRC Crit. Rev. Toxicol.* **1976**, *5*, 271−329.

Received for review January 6, 2004. Revised manuscript received April 29, 2004. Accepted June 4, 2004.

ES0499647