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Assimilation efficiencies and turnover rates of trace elements in marine bivalves: a comparison of oysters, clams and mussels

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Abstract Assimilation efficiencies (AEs) and physiological turnover-rate constants (k) of six trace elements (Ag, Am, Cd, Co, Se, Zn) in four marine bivalves (*Crassostrea virginica* Gmelin, *Macoma balthica* Linnaeus, *Mercenaria mercenaria* Linnaeus, and *Mytilus edulis* Linnaeus) were measured in radiotracer-depuration experiments. Egestion rates of unassimilated elements were highest during the first 24 h of depuration and declined thereafter. Significant egestion of unassimilated Co, however, continued for up to 5 d in *Macoma balthica*, *Mercenaria mercenaria* and *Mytilus edulis*. With the exception of the extremely low values for $^{110\,m}$Ag, ^{109}Cd, and ^{65}Zn in *C. virginica*, physiological turnover-rate constants (k) showed no general pattern of variation among elements, bivalve species or food types, and were relatively invariant. Values from ≤ 0.001 to 0.1 d^{-1} were observed, but excluding those for Co, most values were ≤ 0.04 d^{-1}. In all four species, the AEs of Ag, Am, and Co were generally lower than those of Cd, Se, and Zn. The AEs of Ag, Cd, Se, and Zn in these bivalves are directly related to the proportion of each element in the cytoplasmic fraction of ingested phytoplankton, indicating that >80% of elements in a prey alga's cytoplasm was assimilated. *C. virginica*, *Macoma balthica*, and *Mercenaria mercenaria* assimilated ~36% of the Ag and Cd associated with the non-cytoplasmic (membrane/organelle) fraction of ingested cells in addition to the cytoplasmic fraction. The ratio of AE:k, which is proportional to the consumer–prey trace-element bioaccumulation factor (concentration in consumer:concentration in prey) was generally greater for Cd, Se, and Zn than for Ag, Am, and Co. This ratio was lowest in *Mytilus edulis*, suggesting that this bivalve, the most widely employed organism in global biomonitoring, is relatively inefficient at accumulating important elements such as Ag, Cd, and Zn from ingested phytoplankton.

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Introduction

The trophic transfer of trace elements in marine food webs is increasingly being recognized as an important process influencing metal bioaccumulation and geochemical cycling in marine ecosystems (Fisher and Reinfelder 1995). One of the key parameters needed to quantify trophic transfer is the assimilation efficiency (AE), defined here for metals as the percent of an ingested element that crosses an animal's gut lining. The assimilation of organic carbon in herbivorous bivalves has been thoroughly studied and is fairly well quantified (Winter 1978; Bayne and Newell 1983). It is unlikely, however, that assimilation efficiencies of other elements, particularly trace metals, are the same as that of carbon. Trace elements are distributed heterogeneously among various carbon pools in phytoplankton cells (Fisher et al. 1983b; Reinfelder and Fisher 1991), the principal food of suspension-feeding bivalves. As a result, suspension feeders may accumulate each trace element with a unique assimilation efficiency.

AEs are determined by a number of methods. The traditional mass-balance (Conover and Francis 1973) approach requires accurate measurement of ingestion and egestion, the latter being complicated by loss of fecal matter and associated trace elements during handling (Fisher et al. 1991). These problems are lessened in radiotracer experiments, but care must be taken to use uniformly radiolabeled food to ensure trophic transfer

from all fractions of prey organisms and pulse-feedings to prevent tracer recycling. The use of gamma-emitting radioisotopes in AE determinations permits simultaneous non-destructive measurement of two or more radiotracers in the same sample, and allows the measurement of radiotracers in live animals.

Accurate estimates of consumer AEs and turnover rates, which vary among elements and consumer species, are essential to the development of trace-element accumulation models. Single bivalve–single-element models based on measured uptake and loss rates are fairly good predictors of trace-element accumulation in the field, and can account for seasonal variability in animal physiology (Cutshall 1974; LeFur et al. 1991; Luoma et al. 1992). Further refinement of the uptake rates of such models requires the quantification of trace-element AEs from various food sources, ingestion rates, metal concentration in food particles, and independent measurement of direct accumulation from the dissolved phase. Models thus refined (e.g. Wang et al. 1996) can be used to assess the relative importance of food and water to total bioaccumulation and will aid the interpretation of results from bivalve biomonitoring programs (Phillips 1980; Goldberg et al. 1983; O'Connor 1992).

Recently a major effort was made to determine the assimilation efficiencies of a number of elements in mussels (Wang et al. 1995, 1996; Fisher et al. 1996; Wang and Fisher 1996a, b). This paper presents the results of complementary studies of trace-element assimilation efficiencies in an oyster (*Crassostrea virginica*), mussels (*Mytilus edulis*) and two clams (*Macoma balthica* and *Mercenaria mercenaria*). These bivalves include epifaunal (*C. virginica* and *Mytilus edulis*), and infaunal (*Mercenaria mercenaria*) suspension feeders and an infaunal, facultative deposit feeder (*Macoma balthica*). All these bivalves except *Mercenaria mercenaria* are used extensively in biomonitoring programs in various parts of the world (NOAA 1989). The six elements (Ag, Am, Cd, Co, Se, and Zn) investigated in this study were chosen because they all exhibit elevated concentrations in coastal waters due to human activities and display different patterns of biological and geochemical cycling in aquatic environments. They include essential (Co, Se, Zn) and non-essential (Ag, Am, Cd) elements and hydroxide-complexing (Class A: Am), chloride and sulfur-complexing (Class B: Ag) and borderline (Cd, Zn) metals (Nieboer and Richardson 1980).

Materials and methods

Oysters (*Crassostrea virginica* Gmelin, 4 to 5 cm shell length) and mussels (*Mytilus edulis* Linnaeus, 3 to 3.5 cm shell length) were collected from Long Island Sound, New York (40°54′N; 73° 00′W), clams (*Macoma balthica* Linnaeus, ~1.5 cm shell length) were collected from South San Francisco Bay, California (37°25′N; 122°5′ W), and hard clams (*Mercenaria mercenaria* Linnaeus, 4.5 to 5 cm shell length) from Great South Bay, Long Island, New York (40°45′N; 73°5′W). All bivalves were collected in the winter and early spring. They were scrubbed clean and maintained in aerated glass-fiber-filtered seawater (GFSW, 28‰ S) at 18 °C and fed the athecate prymnesiophyte *Isochrysis galbana* (Clone ISO) or the diatom *Thalassiosira pseudomana* (Clone 3H) for periods of up to 5 d before experiments began. Radiolabeled phytoplankton cells were produced by growing algae in the presence of either ^{110m}Ag, ^{109}Cd and ^{57}Co, or ^{241}Am, ^{75}Se (added as selenite) and ^{65}Zn. The peak gamma-emissions of the three radionuclides in each group can be measured with a NaI(Tl) gamma detector with a minimum of spillover (corrected in all experiments). *I. galbana* and *T. pseudonana* were grown in sterile filtered seawater (SFSW) enriched with *f*/2 (Guillard and Ryther 1962) N, P, Si, and vitamins and *f*/10 trace metals (^{110m}Ag, ^{109}Cd, and ^{57}Co exposure) or *f*/20 trace metals (^{241}Am, ^{75}Se, and ^{65}Zn exposure) minus Cu, Zn and EDTA. Radionuclide additions were 37 kBq l^{-1} (10.5 n*M*) or 61.7 kBq l^{-1} (17.5 n*M*) of ^{110m}Ag from a solution of 0.1 *N* Ultrex HNO_3; 46.3 kBql^{-1} (1.5 n*M*) or 55.5 kBq l^{-1} (1.8 n*M*) of ^{241}Am in 3 *N* Ultrex HNO_3; 123 kBq l^{-1} (58.1 p*M*) or 148 kBq l^{-1}(69.7 p*M*) of ^{109}Cd in 0.1 *N* Ultrex HCl; 123 kBq l^{-1}(7.6 p*M*) or 148 kBq l^{-1} (9.1 p*M*) of ^{57}Co(II) in 0.1 *N* Ultrex HCl; 92.5 kBq l^{-1}(0.35 n*M*) or 111 kBq l^{-1}(0.42 n*M*) of ^{75}Se as selenite in distilled water; and 123 kBq l^{-1}(75 p*M*) or 148 kBq l^{-1}(90 p*M*) of ^{65}Zn in 0.1 *N* Ultrex HCl.

After 4 to 6 d growth, the cells had undergone several divisions (5 to 7) and were considered to be uniformly labeled. Radiolabeled phytoplankton cells were removed from radioactive media (by centrifugation for *Isochrysis galbana* and by Nuclepore filtration for *Thalassiosira pseudonana*) and resuspended in 500 to 600 ml of GFSW (in polycarbonate beakers) at a cell density of 2×10^4 cells ml^{-1}, 0.32 mg dry wt l^{-1} or 0.22 mg algal Cl^{-1} for *I. galbana*, 0.45 mg dry wt l^{-1} or 0.16 mg algal Cl^{-1} for *T. pseudonana*. Resuspended phytoplankton cells were allowed to equilibrate for 45 min before the bivalves were placed individually into the beakers. Each bivalve species was fed radiolabeled monocultures of *I. galbana* and *T. pseudonana* in separate experiments, except for *Crassostrea virginica* which was only fed. *I. galbana*. Replicate experiments included 3 to 7 individuals for *C. virginica*, 5 individuals for *Mercenaria mercenaria*, 5 individuals for *Mytilus edulis* and 3 composites (each having 3 to 4 individuals) for *Macoma balthica*. The bivalves ingested radiolabeled phytoplankton for 40 to 60 min. The potential accumulation of dissolved radioisotope during the pulse-feedings was measured in individuals exposed for 1 h to seawater in which radiolabeled phytoplankton had been previously resuspended for 45 min and then removed by filtration. Radioactivity in the tissues of these individuals was below detection for all radioisotopes.

Following exposure to radiolabeled food, the bivalves were transferred to a recirculating depuration system for 2 wk (Wang et al. 1995). Each individual was held in a 170 ml depuration chamber which exchanged with an aerated reservoir (24 liters of 28‰ GFSW) at a flow rate of 1.8 liters h^{-1}. The water in the reservoir was replaced every week. The bivalves were continuously fed unlabeled phytoplankton (the same species used in the radioactive feedings) at a rate of ≃2% of each individual's dry wt d^{-1}. Whole bivalves were periodically radioassayed non-destructively using a large-well NaI(T1) gamma detector interfaced to a Canberra multichannel analyzer. Thus, the percentage of ingested isotopes retained was followed in the same individual live bivalves during the entire depuration period, eliminating much of the biological variability encountered in physiological studies. Bivalve feces were collected at each sampling time with a pasteur pipette from the bottom of the depuration chambers, and were assayed for radioactivity with a Pharmacia-Wallac LKB gamma counter equipped with a well-type NaI(T1) crystal. The two gamma detectors were intercalibrated and the activity of all samples was determined with radioactive standards for each isotope. Metal egestion rates were calculated as the quotient of fecal radioactivity and the time interval during which feces were produced.

Dissolved radioisotope in the depuration system that could have been excreted directly by the bivalves or released from their feces was below detection during the entire depuration period. Only

^{75}Se and ^{65}Zn were accumulated by radioisotope-free control bivalves in the depuration system. By the end of depuration, however, a maximum of 2% of the activity in bivalves that had ingested radiolabeled phytoplankton could have been due to the accumulation of recycled isotope. The radioactivity in the live bivalves' tissues and feces was measured throughout the depuration periods, which lasted for 11 to 16 d, depending on experiment.

Previous studies suggested that *Macoma balthica* and *Mytilus edulis* complete their digestion and assimilation of ingested elements within 3 to 4 d (Decho and Luoma 1991; Wang et al. 1995). We determined AE values using a curve-stripping method (Riggs 1963) in which bivalve retention data after 4 d were fit to:

$$R_t = AE \cdot e^{-kt} \quad , \tag{1}$$

where R_t = the mean percent of each ingested isotope retained at time t, AE (the assimilation efficiency) = the y-intercept of the ln-transformed regression line of R_t and t, and k = the slope of the regression line, representing the physiological turnover-rate constant of assimilated element. Thus mean ± SD AE and k values were determined using ln-transformed percent retained (R_t) data. The loss-rate constant, k, represents the loss of only that metal accumulated from ingested food. The overall depuration-rate constant, K (see "Discussion"), is the sum of the weighted loss-rate constants of metal accumulated from food and the dissolved phase. Use of Eq. (1) to calculate AE and k assumes that the pool of elements subject to physiological turnover was transported across the bivalves' gut linings and then lost as a consequence of the bivalves' metabolism. It also assumes that this pool of metal was in a single compartment in the bivalves or in multiple compartments having the same characteristic turnover rate. In a few instances the bivalves continued to egest unassimilated radiolabel (in fecal matter) for up to 6 d, and for these experiments (Ag in *Macoma balthica*, Co in *Mercenaria mercenaria*) AEs and ks were calculated using retention data starting at 6 d. This approach should provide consistency among studies involving various bivalve species (which may have different egestion rates) and various elements (which may have a range of gut retention times).

The gamma emissions of ^{110m}Ag were detected at 658 keV, of ^{241}Am at 60 keV, of ^{57}Co at 122 keV, of ^{75}Se at 264 keV, and of ^{65}Zn at 1115 keV. The radioactivity of ^{109}Cd was quantified by measuring ^{109}Ag (daughter product) X-ray emissions at 22 keV; since *Mercenaria mercenaria* shells absorbed a portion of the X-rays associated with the clam's soft tissues, ^{109}Cd activities were corrected using activities measured in dissected individuals. Counting times were adjusted so that propagated counting errors were ≤5%.

Results

The retention of trace elements differed among bivalve species and among elements, indicative of the species-specific and element-specific nature of digestion (Fig. 1). For example, *Mytilus edulis* retained in its tissues substantially less ingested ^{110m}Ag, ^{214}Am, and ^{109}Cd than did the other species. Ingested ^{241}Am was retained to a greater extent in the two clams *Macoma balthica* and *Mercenaria mercenaria* than in the oyster *Crassostrea virginica* or the mussel *Mytilus edulis*, and ingested ^{65}Zn was retained more efficiently by *C. virginica* and *Mercenaria mercenaria* than by *Macoma balthica* and *Mytilus edulis*. Only the pattern of ^{57}Co retention was similar among all species.

Concentrations of ^{110m}Ag, ^{241}Am, ^{109}Cd, and ^{57}Co in feces, determined as Bq defecated h^{-1} bivalve^{-1}, are shown to typify egestion patterns in the different bivalves (Fig. 2). A precipitous decline in egestion rates occurred between 24 and 96 h in all species (Figs. 1 and 2). *Macoma balthica*, the only deposit feeder in the study, released unassimilated label (all elements) more slowly than the other bivalve species. The pattern of ^{57}Co release, after the initial 24 h loss period was complex in all species, a substantial proportion of egested ^{57}Co being collected in the feces of all species between Days 1 and 6 of the egestion period. Detectable amounts of ^{110m}Ag occurred in the feces of all species between 24 and 72 h, suggesting that food was retained in the digestive tract for at least 3 d.

The four bivalve species studied were held under identical experimental conditions, so we can directly compare their rate constants of physiological loss. However, since the depuration period was relatively short for an experiment of this nature, the minimum detectable physiological turnover rate was 0.01 d^{-1}. In addition, since trace-element tissue concentrations could only be followed unambiguously in bivalves that retained sufficient radiolabel, rate constants of loss were not determined for elements with very low (< 15%) assimilation efficiencies (^{110m}Ag-ISO in *Mytilus edulis* and ^{241}Am in *Crassostrea virginica* and *M. edulis*). The physiological turnover-rate constants of ^{110m}Ag, ^{109}Cd, and ^{65}Zn in *C. Virginica* and *Mercenaria mercenaria* and ^{109}Cd in *Macoma balthica* were the lowest measured in this study (Table 1) and, in some cases, not significantly different from zero (*F*-test, Bickel and Doksum 1977). In *Mytilus edulis*, however, the activities of ^{110m}Ag, ^{109}Cd, and ^{65}Zn continued to decrease at detectable rates after 4 d, as did those of ^{110m}Ag and ^{65}Zn in *Macoma balthica* (Fig. 1). Detectable physiological loss of assimilated ^{57}Co and ^{75}Se was observed in all bivalve species (Fig. 1) and Co loss-rates were the highest of all the elements studied (Table 1), coincident with its unique pattern of defecation. With the exception of the extremely low values for ^{110m}Ag, ^{109}Cd, and ^{65}Zn in *C. virginica*, rate constants of loss showed no general pattern of variation among elements, bivalve species, or food types, and in fact were relatively invariant. Values from < 0.01 to 0.1 d^{-1} were observed, but (excluding Co) most values were ≤0.04 d^{-1}.

The AEs of ^{109}Cd, ^{75}Se, ^{65}Zn in all bivalve species were generally higher than those of ^{110m}Ag, ^{241}Am, and ^{57}Co (Table 2). Among bivalve species, AEs varied in a manner consistent with the trends observed in the retention curves. Most notably, *Mytilus edulis* had low AEs of ^{110m}Ag, ^{241}Am, and ^{109}Cd compared with the other bivalves. All bivalves assimilated ^{75}Se with an efficiency > 70% and ^{65}Zn with an efficiency of > 60%.

Trace-element AEs generally increased with the proportion of each element in the cytoplasmic fraction of ingested *Isochrysis galbana* cells (Fig. 3). Positive relationships between the AE and percent of each element in the alga's cytoplasm (cyto) were found for all six elements in *Mytilus edulis* ($AE = 0.86 \times \%\text{cyto} - 2.5, r^2 = 0.90$) and for ^{110m}Ag and ^{109}Cd in *Crassostrea virginica*, *Macoma balthica*, and *Mercenaria mercenaria*

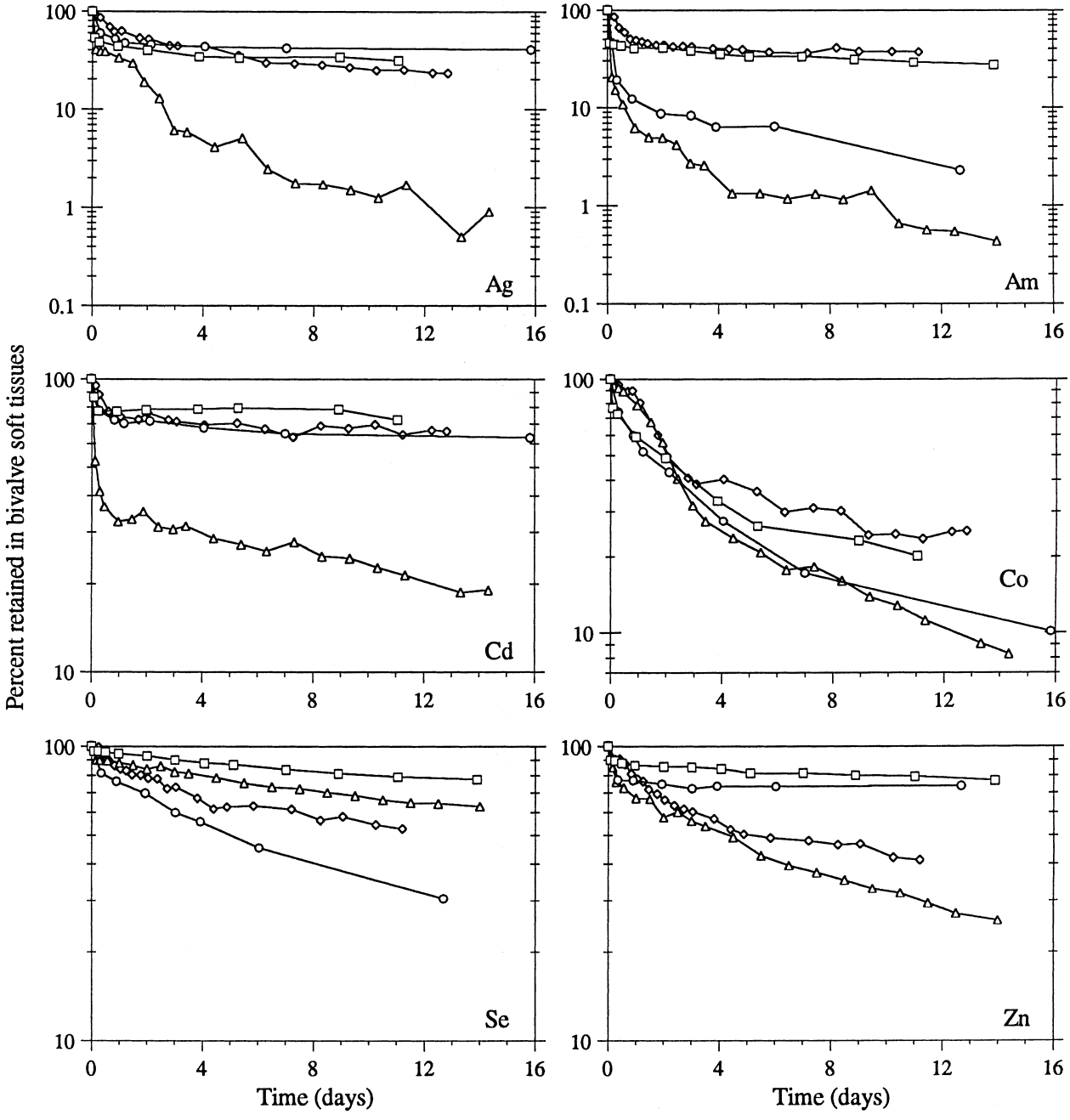

Fig. 1 *Crassostrea virginica* (○), *Macoma balthica* (◇), *Mercenaria mercenaria* (□) and *Mytilus edulis* (△). Retention of Ag, Am, Cd, Co, Se, and Zn in soft tissues of bivalves fed radiolabeled *Isochrysis galbana*. Values are mean percentages of 3 to 7 replicates

(AE = 0.85 × %cyto + 36, $r^2 = 0.71$; Fig 3). Both ^{75}Se and ^{65}Zn were enriched in the cytoplasmic fraction of *I. galbana* cells (Fig. 3) and both elements were very efficiently assimilated by all four bivalves (Table 2). ^{57}Co was also enriched in *I. galbana*'s cytoplasm, but the AEs of ^{57}Co in *C. virginica*, *M. mercenaria*, and *Mytilus edulis*, which were within 12% of each another, were 30 to 40% lower than the percentages of ^{57}Co in the cytoplasmic fraction of ingested algae.

Discussion

Food-processing rates in the present study tended to be element- and bivalve-species-specific. Very little ^{241}Am was retained longer than 1 d by *Mytilus edulis* (nearly all the ingested ^{241}Am was egested in the first rapid pulse), as was shown in other experiments with this species (Wang et al. 1995). *Crassostrea virginica*, which like

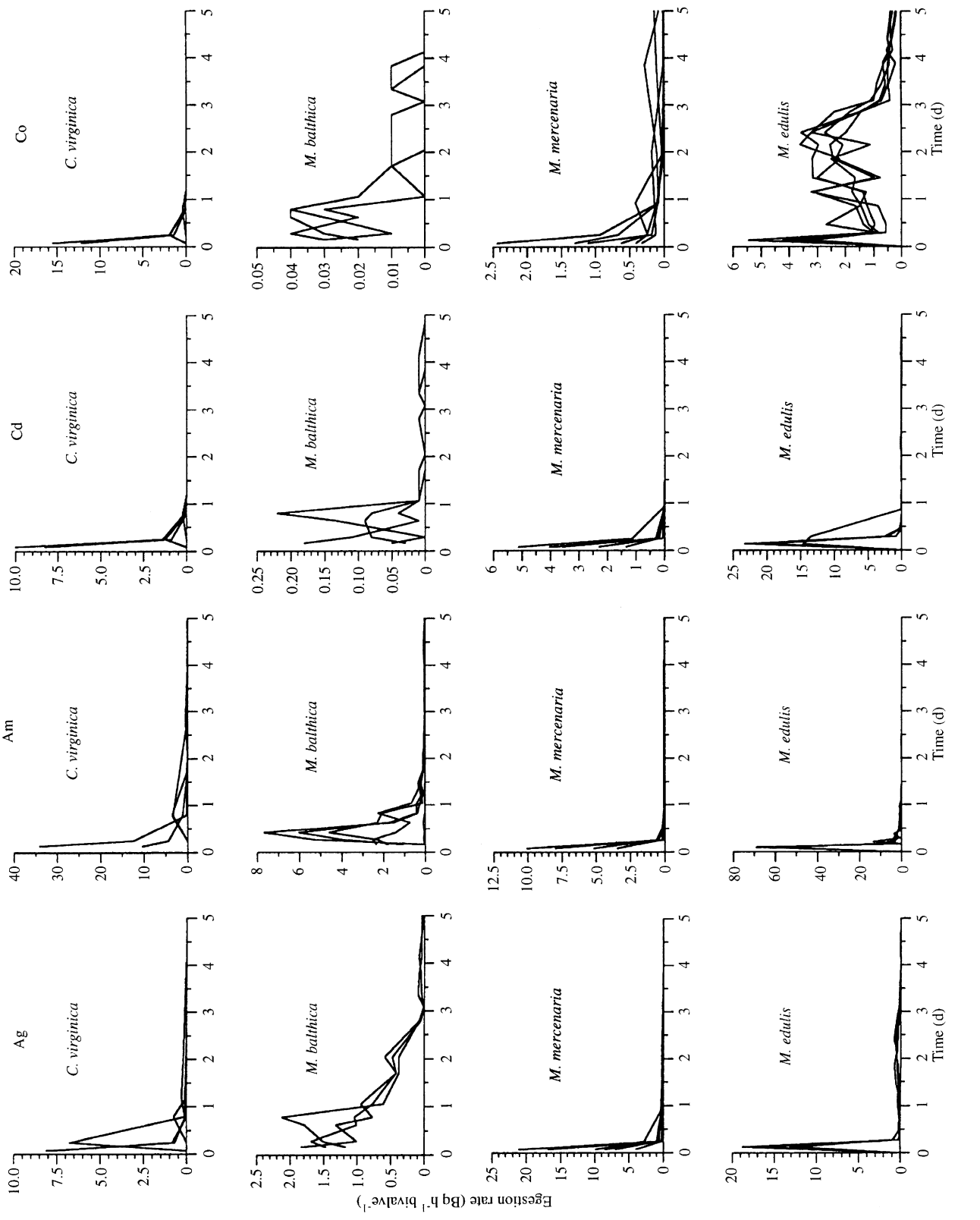

Fig. 2 *Crassostrea virginica*, *Macoma balthica*, *Mercenaria mercenaria* and *Mytilus edulis*. Trace-metal (^{110m}Ag, ^{241}Am, ^{109}Cd, and ^{57}Co) egestion rates in individual bivalves fed radiolabeled *Isochrysis galbana*

M. edulis has a low Am AE (11%), also egested nearly all unassimilated Am in 1 d, although low, but detectable rates of Am egestion were measured for up to 3 d. Little ^{241}Am occurred in the feces of *Macoma balthica* or *Mercenaria mercenaria* after 1 d, but in contrast to

Table 1 *Crassostrea virginica*, *Macoma balthica*, *Mercenaria mercenaria*, and *Mytilus edulis*. Physiological turnover-rate constants (k, d^{-1}; mean ± SD) of assimilated trace elements in oysters, hard clams and mussels. Rate constants were calculated as slopes of the ln-linear radiotracer-retention curves. [*Numbers in parentheses* biological half-lives (ln2 ÷ k, d); *Iso Isochrysis galbana*; *3H Thalassiosira pseudonana*; *nd* not determined]

Element, food	*C. virginica*	*M. balthica*	*M. mercenaria*	*M. edulis*
Ag				
Iso	< 0.01[a] (> 70)	0.04 ± 0.003[b] (17)	0.01 ± 0.0007 (70)	–[c]
3H	nd	0.09 ± 0.005[b] (8)	0.02 ± 0.004 (35)	0.04 ± 0.02 (17)
Am				
Iso	–[c]	< 0.01[a] (> 70)	0.02 ± 0.002 (35)	–[c]
3H	nd	0.05 ± 0.01 (14)	nd	–[c]
Cd				
Iso	< 0.01[a] (> 70)	< 0.01[a] (> 70)	0.01 ± 0.003 (70)	0.05 ± 0.003 (14)
3H	nd	0.01 ± 0.007 (70)	< 0.01[a] (> 70)	0.02 ± 0.004 (35)
Co				
Iso	0.08 ± 0.004 (9)	0.08 ± 0.02 (9)	0.05 ± 0.004[b] (14)	0.1 ± 0.02 (7)
3H	nd	0.09 ± 0.02 (8)	0.04 ± 0.02[b] (17)	0.2 ± 0.02 (3.5)
Se				
Iso	0.07 ± 0.0008 (10)	0.03 ± 0.01 (23)	0.01 ± 0.004 (70)	0.02 ± 0.007 (35)
3H	nd	0.03 ± 0.02 (23)	nd	0.05 ± 0.004 (14)
Zn				
Iso	< 0.01[a] (> 70)	0.04 ± 0.005 (17)	0.01 ± 0.002 (70)	0.07 ± 0.006 (10)
3H	nd	0.04 ± 0.004 (17)	nd	0.05 ± 0.006 (14)

[a] Values not significantly different from zero (F-test)
[b] Determined after 6 d depuration
[c] Rate constant omitted because of low assimilation efficiencies

Table 2 *Crassostrea virginica*, *Macoma balthica*, *Mercenaria mercenaria*, and *Mytilus* spp. (*M. edulis* and *M. galloprovincialis*). Assimilation efficiencies (%, mean ± SD) of trace elements in bivalves, calculated as intercepts of physiological turnover portions of radiotracer-retention curves (*Iso Isochrysis galbana*; *3H Thalassiosira pseudonana*) Assimilation efficiencies of mussels fed *T. pseudonana* are geometric means of those from five different studies shown in Table 3

Element, food	*C. virginica*	*M. balthica*	*M. mercenaria*	*Mytilus* spp.
Ag				
Iso	44 ± 7.9	38 ± 1.0[a]	35 ± 6.9	13 ± 5.8
3H	nd	49 ± 1.1[a]	22 ± 3.5	22 ± 16
Am				
Iso	11 ± 12	41 ± 11	38 ± 9.3	2.9 ± 0.4
3H	nd	33 ± 5.5	nd	4.0 ± 1.4
Cd				
Iso	69 ± 8.6	69 ± 2.3	83 ± 17	37 ± 5.2
3H	nd	88 ± 9.9	66 ± 13	41 ± 27
Co				
Iso	34 ± 10.1	53 ± 0.2	34 ± 4.9[a]	46 ± 6.7
3H	nd	45 ± 4.1	29 ± 2.4	27 ± 12
Se				
Iso	70 ± 6.2	78 ± 11	92 ± 1.6	86 ± 1.8
3H	nd	74 ± 7.2	nd	74 ± 7.8
Zn				
Iso	73 ± 8.2	64 ± 4.8	86 ± 1.3	62 ± 10
3H	nd	50 ± 5.1	nd	39 ± 13

[a] Determined after 6 d depuration

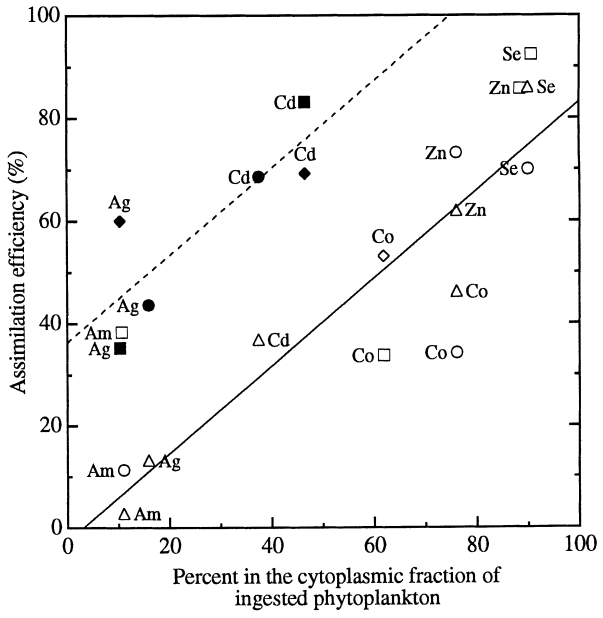

Fig. 3 *Crassostrea virginica* (○), *Macoma balthica* (◇), *Mercenaria mercenaria* (□) and *Mytilus edulis* (△). Trace-element assimilation efficiencies (AE) in bivalves compared with percent of each element in cytoplasmic fraction of ingested *Isochrysis galbana*. Cytoplasmic (cyto) fractionation was determined according to Reinfelder and Fisher (1994) [*Dashed line* relationship for Ag and Cd in *C. Virginica*, *Macoma balthica*, and *Mercenaria mercenaria* (*black symbols*; $AE = 0.85 \times cyto + 36$, $r^2 = 0.71$); *continuous line* relationship for all elements in *Mytilus edulis* alone ($AE = 0.86 \times cyto - 2.5$, $r^2 = 0.90$)] Cell fractionation of Am, Se, and Zn was not determined in *Macoma balthica* experiments

C. virginica and *Mytilus edulis*, both clams assimilated this element with moderate efficiencies (33 to 41%). Rapid gut-passage of Am may account for the low Am AEs in *C. virginica* and *M. edulis*, whereas intensive processing of the Am-enriched fraction of the phytoplankton food may explain the more efficient assimilation of this element by the two clams. Longer gut-retention enhanced the assimilation of ^{51}Cr and ^{109}Cd in *Macoma balthica* (Decho and Luoma 1991, 1994, 1996). In this study, little ^{109}Cd was found in the bivalves' feces after 1 d. Unassimilated Cd appears to be efficiently egested during the early phase of digestion, but ingested Cd that is retained longer than 1 d is nearly all assimilated. The retardation of Co movement through the alimentary canals of *M. balthica*, *Mercenaria mercenaria* and *Mytilus edulis* may be the result of Co oxidation (Lee and Fisher 1993), and the precipitation of Co hydroxides in bivalves' guts as has been observed for Fe in mussels (Hobden 1969) and oysters (George et al. 1978). By contrast, the gut-passage rate of Ag in all four bivalves may have been slowed by the binding of Ag to sulfide groups in the digestive diverticula, as has been observed in mussels (George et al. 1986).

Metal–bivalve steady-state is attained in some species only after more than a year of exposure (e.g. *Anodonta grandis*: Tessier et al. 1993). The extremely slow turnover rates of some elements in this study (e.g. Ag, Cd, Zn) suggest that assimilated radiotracers were able to accumulate in the bivalves' slowly exchanging pools even through radiolabeled algae were fed to them for only 40 to 60 min. The generally narrow range of k values found in this study (most between ≤0.01 to 0.04 d^{-1}) for all elements except Co indicates that physiological turnover rates may not be particularly variable among different elements or bivalves. However, further study is needed on the depuration kinetics of elements with low assimilation efficiencies (e.g. Ag and Am) and those subject to redox chemistry (e.g. Co). For example, recent work by Fisher et al. (1996) suggests that the physiological loss-rate constants of Ag and Am in *Mytilus galloprovincialis* range from 0.06 to 0.11 d^{-1}.

As found for planktonic copepods (Reinfelder and Fisher 1991) and bivalve larvae (Reinfelder and Fisher 1994), trace-element AEs in adult bivalves cover almost the entire range of possible values (3 to 92%). Nonessential elements (with the exception of Cd) are assimilated least efficiently, while essential elements (with the exception of Co) are assimilated with high efficiencies (Table 2). The variability of trace-element AE measurements can be assessed by comparing AEs in one consumer fed the same food. Table 3 shows the AEs of six trace elements in mussels (genus *Mytilus*) fed the diatom *Thalassiosira pseudonana* determined in five different studies. Generally there was good agreement between the AEs of metals determined in this study and previous findings. The two exceptions are for Ag and Cd, both of which displayed much higher AEs in this study than in all other studies using comparable protocols; reasons for these discrepancies are not apparent.

Like those in copepods and bivalve larvae, trace-element AEs in adult bivalves are similar in individuals fed *Isochrysis galbana* or *Thalassiosira pseudonana*, both

Table 3 Mussels (*Mytilus edulis* and *M. galloprovincialis*) fed the diatom *Thalassiosira pseudonana*. Values are trace-element assimilation efficiencies (%) (*nd* not determined)

Species	Element						Source
	Ag	Am	Cd	Co	Se	Zn	
M. edulis	50	3	96	37	75	43	Present study
	8	3	35	22	78	26	Wang et al. (1995)
	17	5	34	38	72	44	Wang and Fisher (1996a)
	19	3	21, 26, 53	27	63, 84	44, 48, 50	Wang and Fisher (1996b)
M. galloprovinciallis	15	6	22	9	nd	15	Fisher et al. (1996)

high-quality algal foods. Mussels fed diatoms or the chlorophyte *Dunaliella tertiolecta* also had similar trace-element AEs (Fisher and Teyssié 1986). Passage of the siliceous roughage associated with a diet of diatoms (*T. pseudonana*) apparently does not significantly affect trace-element AEs in adult bivalves. Wang and Fisher (1996a) recently found that food composition affects the AEs of trace elements in mussels when poor-quality food algae are compared with nutritious species. In their study, carbon AEs (one measure of food quality) were well correlated with the AEs of Cd, Se, and Zn, but not with the AEs of Ag, Am, or Co.

Suspension-feeding herbivores generally do not absorb Ag, Am, or Co efficiently from ingested phytoplankton, but our results suggest that some bivalves have relatively high AEs for these metals. Euphausiids (Fisher et al. 1983a), copepods (Fisher et al. 1991; Reinfelder and Fisher 1991), oyster and hard clam larvae (Reinfelder and Fisher 1994), and adult mussels and oysters (Table 2) assimilate $<11\%$ of ingested Am. The clams *Macoma balthica* and *Mercenaria mercenaria*, however, retain $\sim 40\%$ of ingested Am (Table 2). Among the bivalves examined here, *Macoma balthica*, a facultative deposit feeder, had the highest AEs for both Ag and Co. Depuration rates of ingested ^{110m}Ag and ^{60}Co in the deposit-feeding clam *Scrobicularia plana* (Amiard-Triquet and Amiard 1976; Amiard 1978) indicated Ag and Co AEs of $\sim 80\%$. *M. balthica* and *Mercenaria mercenaria* are infaunal, taxonomically distinct from oysters and mussels, and may have digestive systems which are modified by the large quantities of detritus and sediment in their food.

The AEs of Se in the four bivalves we examined, like those in other marine herbivores (Fisher and Reinfelder 1991; Reinfelder and Fisher 1994), are very high ($>70\%$). As a result, a significant portion of Se body burdens in suspension-feeding bivalves is very probably due to accumulation from ingested food, as shown for *Macoma balthica* in San Francisco Bay (Luoma et al. 1992).

The AE and cell fractionation results in Fig. 3 support the hypothesis that elements enriched in the cytoplasmic fraction of ingested phytoplankton food are assimilated more efficiently by aquatic herbivores than elements that are not concentrated in this fraction. All six elements are assimilated by *Mytilus edulis*, with AEs that increase in proportion to the percent of these elements in the cytoplasmic fraction of ingested *Isochrysis galbana*, a pattern which also applies to Se and Zn in *Crassostrea virginica* and *Mercenaria mercenaria*. This is consistent with the work of Wang and Fisher (1996a) who found significant positive relationships between the AEs of seven trace elements in *Mytilus edulis* and the cytoplasmic distribution of the elements in five out of seven species of phytoplankton studied. In *C. virginica*, *Macoma balthica*, and *Mercenaria mercenaria*, the AEs of Ag and Cd also correlate with cytoplasmic enrichment, but the relationship is offset toward higher AEs from that of *Mytilus edulis* by $\simeq 36\%$ (Fig. 3). These three bivalves can apparently assimilate a significant portion of the Ag and Cd associated with the non-cytoplasmic fraction of ingested algae. Enhanced assimilation as a result of prolonged gut retention may explain the greater assimilation of Ag, Cd, and Am (Am only in clams) in excess of what was expected from the partitioning of those elements in the cytoplasmic fraction of ingested phytoplankton cells (Fig. 3). Ag and Cd, two nonessential metals, are likely to become associated with cellular proteins due to their propensity to bind with S. Efficient assimilation of Ag and Cd in *C. virginica*, *Macoma balthica,* and *Mercenaria mercenaria* may therefore result from more thorough digestion of non-cytoplasmic, membrane proteins in these bivalves than in *Mytilus edulis* or the larvae of *C. virginica* and *Mercenaria mercenaria* (Reinfelder and Fisher 1994). The slope of the line relating the AEs and cytoplasmic fractionation of Ag and Cd in *C. virginica*, *Macoma balthica*, and *Mercenaria mercenaria* is nearly identical to that for *Mytilus edulis*, suggesting that the process of assimilation of elements from the cytoplasmic fraction of algal prey is similar in all four species.

The importance of assimilation and physiological loss to the overall accumulation of trace elements in bivalves can be assessed by resolving the steady-state concentrations (C_{ss}) of trace elements in bivalves into individual uptake and loss pathways according to the following equation (Thomann 1981; Luoma et al. 1992; Wang et al. 1996):

$$C_{ss} = [a_w \cdot \mathrm{FR} \cdot C_w + AE \cdot \mathrm{I} \cdot C_{prey}]/(K + G) \ , \qquad (2)$$

where a_w = the absorption efficiency of a trace element from the dissolved phase, FR = the filtration rate, C_w and C_{prey} = the trace-element concentrations in the water and prey, respectively, I = the weight-specific ingestion rate, AE = the assimilation efficiency, K = the overall depuration-rate constant, and G = the growth-rate constant. The overall depuration-rate constant (K) is the loss constant for elements accumulated from all sources, including the rate constant for loss following uptake from the dissolved phase and the physiological turnover-rate constant for elements accumulated from food (K). Efflux-rate constants in *Mytilus edulis* have been experimentally shown to be comparable for metals obtained form food and dissolved pathways (Fisher et al. 1996; Wang et al. 1996).

Eq. (2) implies that, all else being equal, the consumer–prey bioaccumulation factor ($BAF = C_{SS} : C_{prey}$), is proportional the ratio of AE:k (Hattum et al. 1989). The BAFs of Ag, Cd, and Zn in *Mytilus edulis*, indicated by the low AE:k ratios in Table 4, are low. Compared with other bivalves, this species, the most widely employed organism in global biomonitoring, is relatively inefficient in bioaccumulating important elements such as Ag, Cd, and Zn from its food. This prediction is consistent with existing field data from biomonitoring programs. For example, O'Connor (1992) reported that Ag, Cd, and Zn concentrations in oysters (*Crassostrea virginica*) were on average 15, 1.8, and 42 times higher,

Table 4 *Crassostrea virginica*, *Macoma balthica*, *Mercenaria mercenaria*, and *Mytilus edulis*. Oysters, hard clams and mussels fed *Isochrysis galbana*. Ratio of assimilation efficiencies and physiological loss-rate constants (AE:*k*)

Element	*C. virginica*	*M. balthica*	*M. mercenaria*	*M. edulis*
Ag	4400	950	3500	550[a]
Am	–[c]	4100	1900	60[b]
Cd	6900	6900	8300	740
Co	425	663	680	460
Se	1000	2600	9200	4300
Zn	7300	1600	8600	886

[a] Calculated for mussels fed *Thalassiosira pseudonana*
[b] Calculated from data of Fisher et al. (1996) for *Mytilus galloprovincialis*
[c] Ratio omitted since a reliable *k* could not be determined for Am in *C. virginica*

respectively, than those in mussels collected from Long Island Sound as part of the National Status and Trends (NS & T) Program. In contrast, Se concentrations measured in the NS & T program were 1.4 times higher in mussels than in oysters, also consistent with predictions from Table 4. *Macoma balthica* and *Mercenaria mercenaria* are expected to accumulate more contaminant radioisotopes (e.g. ^{60}Co, ^{241}Am) from ingested phytoplankton than oysters or mussels, although Co was not strongly bioaccumulated by any of the bivalves in this study. Such predictions assume phytoplankton food to be the principal source of uptake and, as such, do not address the usefulness of bivalves that accumulate contaminant trace elements mainly from the dissolved phase as biomonitors of environmental contamination. They also do not take into consideration the effects of variable environmental factors on trace-element accumulation rates. The high bioaccumulation of Cd predicated by its high AE:*k* ratio in *Macoma balthica*, for example, is not observed in nature (Brown and Luoma 1995); geochemical processes such as metal sulfide formation may reduce Cd availability for this and other infaunal bivalves.

Interspecies comparisons of trace-element bioaccumulation have been rare to date. Such studies have the advantage of eliminating the potentially significant but often unrecognized variability associated with different exposure and depuration conditions. Differential bioaccumulation among marine consumers could influence trace-element cycling, including trophic transfer, and the comparative approach may prove useful in the identification of species that accumulate these ubiquitous anthropogenic contaminants the most, and the inter-pretation of trends in marine contamination.

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References

Amiard J-C (1978) Modalites de la contamination d'une chaine trophique marine benthique par l'argent 110m 4. Influence du mode de contamination sur l'élimination du radionucléide. J exp mar Biol Ecol 34: 215–225

Amiard-Triquet C, Amiard J-C (1976) Etude expérimental du transfert du cobalt-60 dans une chaine trophique marine benthique. Helgoländer wiss Meersunters 27: 283–297

Bayne BL, Newell RC (1983) Physiological energetics of marine molluscs. In: Salenddin ASM, Wilbur KM (eds) The Mollusca. Vol. 4. Academic Press. London, pp 407–515

Bickel PJ, Doksum KA (1977) Mathematical statistics. Holden–Day, San Francisco

Brown CL, Luoma SN (1995) Use of the euryhaline bivalve *Potamocorbula amurensis* as a biosentinel species to assess trace metal contamination in San Fancisco Bay. Mar Ecol Prog Ser 124: 129–142

Conover RJ, Francis V (1973) The use of radioactive isotopes to measure the transfer of materials in aquatic food chains. Mar Biol 18: 272–283

Cutshall N (1974) Turnover of zinc-65 in oysters. Hlth Phys 26: 327–331

Decho AW, Luoma SN (1991) Time courses in the retention of food material in the bivalves *Potamocorbula amurensis* and *Macoma balthica*: significance to the absorption of carbon and chromium. Mar Ecol Prog Ser 78: 303–314

Decho AW, Luoma SN (1994) Humic and fulvic acids: sink or source in the availability of metals to the marine bivalves *Macoma balthica* and *Potamocorbula amurenis?* Mar Ecol Prog Ser 108: 133–145

Decho AW, Luoma SN (1996) Flexible digestion strategies and trace metal assimilation in marine bivalves. Limnol Oceanogr 41: 568–572

Fisher NS, Bjerregaard P, Fowler SW (1983a) Interactions of marine plankton with transuranic elements. 3. Biokinetics of americium in euphausiids. Mar Biol 75: 261–268

Fisher NS, Burns KA, Cherry RD, Heyraud M (1983b) Accumulation and cellular distribution of ^{241}Am, ^{210}Po, and ^{210}Pb in two marine algae. Mar Ecol Prog Ser 11: 233–237

Fisher NS, Nolan CV, Fowler SW (1991) Assimilation of metals in marine copepods and its biogeochemical implications. Mar Ecol Prog Ser 71: 37–43

Fisher NS, Reinfelder JR (1991) Assimilation of selenium in the marine copepod *Acartia tonsa* studied with a radiotracer ratio method. Mar Ecol Prog Ser 70: 157–164

Fisher NS, Reinfelder JR (1995) The trophic transfer of metals in marine systems. In: Tessier A, Turner DR (eds) Metal speciation and bioavailability in aquatic systems. John Wiley & Sons, Chichester, pp 363–406

Fisher NS, Teyssié J-L (1986) Influence of food composition on the biokinetics and tissue distribution of zinc and americium in mussels. Mar Ecol Prog Ser 28: 197–207

Fisher NS, Teyssié J-L, Fowler SW, Wang WX (1996) The accumulation and retention of metals in mussels from food and water: a comparison of field and laboratory conditions. Envir Sci Technol 30: 3232–3242

George SG, Pirie BJS, Calabrese A, Nelson DA (1986) Biochemical and ultrastructural observations of long-term silver accumulaion in the mussel, *Mytilus edulis*. Mar envirl Res 18: 255–265

George SG, Pirie BJS, Cheyne AR, Coombs TL, Grant PT (1978) Detoxication of metals by marine bivalves: an ultrastructural study of the compartmentaion of copper and zinc in the oyster *Ostrea edulis*. Mar Biol 45: 147–156

Goldberg ED, Koide M, Hodge V, Flegal AR, Martin J (1983) U.S. mussel watch: 1977–1978 results on trace metals and radionuclides. Estuar cstl Shelf Sci 16: 69–93

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8: 229–239

Hattum B, van de Voogt P, van den Bosch L, van Straalen NM, Joosse ENG (1989) Bioaccumulation of cadmium by the freshwater isopod *Asellus aquaticus* (L.) from aqueous and dietary sources. Envir Pollut 62: 129–151

Hobden DJ (1969) Iron metabolism in *Mytilus edulis*. II. Uptake and distribution of radioactive iron. J mar Res 49: 661–668

Lee B-G, Fisher NS (1993) Microbially mediated cobalt oxidation in seawater revealed by radiotracer experiments. Limnol Oceanogr 38: 1593–1602

LeFur J, Germain P, Baron Y (1991) Modelling in situ mussel ruthenium-106 concentration dynamics due to fluctuating seawater radionuclide concentrations. Mar Ecol Prog Ser 75: 67–78

Luoma SN, Johns C, Fisher NS, Steinberg NA, Oremland RS, Reinfelder JR (1992) Determination of selenium bioavailability to a benthic bivalve from particulate and solute pathways. Envir Sci Technol 26: 485–491

Nieboer E, Richardson DH (1980) The replacement of the nondescript term 'heavy metals' by a biological and chemically significant classification of metal ions. Envir Pollut (Ser B) 1: 3–26

NOAA (1989) A summary of data on tissue contamination from the first three years (1986–1988) of the mussel watch project. NOAA natn mar Fish Serv tech Memo US Dep Comm (NOS OMA) 49: 1–151

O'Connor TP (1992) Recent trends in coastal environment quality: results from the five years of the NOAA mussel watch project. US Department of Commerce, National Oceanic and Atmospheric Administration, Washington, DC, p. 46

Phillips JDH (1980) Quantitative aquatic biological indicators. Applied Scence Publishers, London

Reinfelder JR, Fisher NS (1991) The assimilation of elements ingested by marine copepods. Science, NY 251: 794–796

Reinfelder JR, Fisher NS (1994) The assimilation of elements ingested by planktonic bivalve larvae. Limnol Oceanogr 39: 12–20

Riggs DS (1963) The mathematical approach to physiological problems. Williams & Wilkens Co., Baltimore

Tessier A, Couillard PG, Campbell PGC, Auclair JC (1993) Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis*. Limnol Oceanogr 38: 1–17

Thomann RV (1981) Equilibrium model of fate of microcontaminants in diverse aquatic food chains. Can J Fish aquat Sciences 38: 280–296

Wang W-X, Fisher NS (1996a) Assimilation of trace elements and carbon by the mussel, *Mytilus edulis*: effects of food composition. Limnol Oceanogr 41: 197–207

Wang W-X, Fisher NS (1996b) Assimilation of trace elements by the mussel *Mytilus edulis*: effects of diatom chemical composition. Mar Biol 125: 715–724

Wang W-X, Fisher NS, Luoma SN (1995) Assimilation of trace elements ingested by the mussel *Mytilus edulis*: effects of algal food abundance. Mar Ecol Prog Ser 129: 165–176

Wang W-X, Fisher NS, Luoma SN (1996) Kinetic determinations of trace element bioaccumulation in the mussel, *Mytilus edulis*. Mar Ecol Prog Ser 140: 91–113

Winter JE (1978) A review on the knowledge of suspension-feeding in the lamellibranchiate bivalves, with special reference to artificial aquaculture systems. Aquaculture, Amsterdam 13: 1–33