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Assimilation and regeneration of trace elements by marine copepods

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Abstract

Assimilation efficiencies (AE) of five trace elements (Am, Cd, Co, Se, and Zn) and carbon by neritic copepods (*Acartia tonsa* and *Temora longicornis*) feeding at different food concentrations and on different food types (diatoms, green algae, flagellates, dinoflagellates, and Fe oxides) were measured with radiotracer techniques. Food concentration had little influence on AEs of C, Cd, Co, and Se within a range of 16–800 μg C liter^{-1}. AEs of Am and Zn were highest at low food concentrations (16–56 μg C liter^{-1}) but remained relatively constant when food levels exceeded 160 μg C liter^{-1}. Different algal diets had no major influence on AEs, which generally were in the order Cd > Se > Zn > Co > Am. Metals (Cd, Co, and Zn) were assimilated from Fe oxides with 50% less efficiency than from algal cells. Element regeneration into the dissolved phase was a significant route for the release of ingested elements by copepods and increased with increased food concentration. Element regeneration rates for Cd, Se, and Zn were comparable to the regeneration rates of major nutrients such as P (30–70% daily). Retention half-times of elements in decomposing fecal pellets ranged from <1 d (Cd) to >10 d (Am). The efficient assimilation and regeneration of Cd, Se, and Zn can significantly lengthen the residence time of these elements in ocean surface waters.

Biological processes can profoundly influence the geochemical behavior of trace elements in surface waters (Fowler and Knauer 1986). Phytoplankton concentrate many trace elements out of seawater (concentration factors often exceeding 10^3) and rapidly reach equilibrium with their aqueous environments. Trace elements can be removed from surface waters on sinking biogenic debris, including phytoplankton aggregates and zooplankton fecal pellets. Elements that enter the ocean via atmospheric deposition and are efficiently removed from the water column by such processes typically exhibit scavenged-type vertical profiles, in which surface concentrations exceed concentrations at depth (Whitfield and Turner 1987). Elements that are assimilated by grazers, however, enter into the organic cycles of the ocean and are recycled in surface waters. These elements generally have longer residence times in surface waters and exhibit nutrient-type vertical profiles, showing surface depletion and middepth maxima (Whitfield and Turner 1987). Furthermore, biologically mediated regeneration of trace elements into the dissolved phase may significantly increase their residence times in surface waters. The assimilation of elements from phytoplankton by marine zooplankton is therefore important in determining the fate of elements in ocean surface waters.

The extent of metal bioconcentration by marine phytoplankton from ambient seawater has been described (Fisher and Reinfelder 1995). Generally, it has been observed that essential elements exhibit greater penetration into the cytoplasm of algal cells than do nonessential elements, which typically remain adsorbed onto cell surfaces (Fisher et al. 1983*c*; Reinfelder and Fisher 1991). The rates and routes of trace metal release from biogenic particles into the dissolved phase have also been studied for decomposing phytoplankton (Lee and Fisher 1992*a*, 1993; Fisher and Wente 1993) and zooplankton debris (Lee and Fisher 1992*b*, 1994; Reinfelder et al. 1993). Elemental release from decomposing biogenic debris is governed by desorption, physical leaching, and bacterial degradation (Lee and Fisher 1992*b*) and can be enhanced by the processes of ingestion, digestion, and defecation during zooplankton grazing (Lee and Fisher 1994; Hutch-

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Table 1. Algal diets used in food quality feeding experiments. Clonal designations from the National Marine Phytoplankton Culture Collection (Bigelow Laboratory, Maine). Cell dry weights were measured by the ammonium formate-rinsing technique.

	Clone	Division	Dry wt (pg cell^{-1})
Chlorella autotrophica	CCMP243	Chlorophyceae	17.9±0.7
Phaeodactylum tricornutum	CCMP630	Bacillariophyceae	31.8±0.9
Prorocentrum minimum	CCMP1329	Dinophyceae	1,290±163
Rhodomonas salina	CCMP1319	Cryptophyceae	88.5±3.3
Tetraselmis levis	CCMP896	Prasinophyceae	164±8.8
Tetraselmis maculata	CCMP897	Prasinophyceae	150±22.3
Thalassiosira pseudonana	CCMP1335(3H)	Bacillariophyceae	22.5±1.2

ins and Bruland 1994). After assimilation, regeneration of metals by marine zooplankton via physiological turnover is another pathway for the release of metals from particulate phases into the dissolved phase, but it has received little study.

In marine copepods, assimilation of trace elements is governed by the cytoplasmic distribution in prey cells (Reinfelder and Fisher 1991; Hutchins et al. 1995). Elements in the cytoplasm seem to be completely assimilated by copepods, whereas elements bound to cell surfaces are unassimilated and packaged into fecal pellets. This "liquid" digestive strategy appears to operate in marine bivalve larvae as well (Reinfelder and Fisher 1994). The short gut residence time typical of these herbivores, together with the churning of prey's soluble fractions, may allow them to process ingested food materials rapidly. This digestion strategy may represent one type of optimal feeding, in which digestion and assimilation are conserved because of the high energetic costs associated with these physiological processes (Kiørboe et al. 1985). Copepods may therefore respond to changes in their food supply with a change in feeding activity (e.g. selectivity, ingestion rate) that minimizes their energy expenditure while meeting nutritional requirements.

No reports in the literature compare metal assimilation in zooplankton from diverse phytoplankton species, nor are there studies which examine the effects of algal cell density on the trophic transfer of metals to zooplankton. We therefore conducted a series of experiments to examine the effects of algal diet—both quality and quantity—on the assimilation of five trace elements plus carbon in marine copepods. We also determined rates of metal regeneration into the dissolved phase following assimilation in the animals. Copepods were fed inorganic particles (iron oxides) and seven species of phytoplankton representing diverse algal classes, and the partitioning of carbon and the trace elements among different pools was measured. The trace elements were studied by means of gamma-emitting radiotracers (^{241}Am, ^{109}Cd, ^{57}Co, ^{75}Se, and ^{65}Zn). Among these elements, Co, Se, and Zn are essential to living organisms, Am is used as a representative particle-reactive metal with no known biological function, and Cd may substitute for Zn when the latter becomes deficient in marine diatoms (Price and Morel 1990).

Materials and methods

Adult copepods, *Acartia tonsa* and *Temora longicornis,* in a ratio of ~80% to 20%, were collected from Stony Brook Harbor, Long Island, New York. Copepods were kept in the laboratory at 15°C in 35‰ glass-fiber-filtered seawater (collected in the Atlantic Ocean 8 km off Southampton, Long Island) for 1 d and fed algal food of the same species used during radiotracer feeding experiments.

Radiolabeling phytoplankton—Seven species of phytoplankton (Table 1) representing five algal divisions were kept in unialgal, clonal cultures with f/2 medium (Guillard and Ryther 1962). Cells at late log growth phases were collected on 1-μm polycarbonate membranes and resuspended into 0.2-μm-filtered 35‰ seawater. Because *Rhodomonas salina* could not be quantitatively recovered off filters, the cells were collected by centrifugation at 2,000 × *g* for 10 min.

Media for the trace element experiments (200 ml) were enriched with f/2 levels of N, P, Si, vitamins, and f/20 trace metals without additions of Cu, Zn, and EDTA. For ^{14}C-^{51}Cr exposure experiments, culture media were enriched with f/2 levels of all nutrients. The initial cell concentration in the radiotracer uptake media was generally 5×10^4 cells ml^{-1}.

For each algal species, three flasks were amended with either ^{109}Cd and ^{57}Co, or ^{241}Am, ^{75}Se, and ^{65}Zn, or ^{14}C and ^{51}Cr. Radioisotope additions, made in microliter amounts, were 74 kBq of ^{241}Am (in 3 N HNO_3) to yield a final concentration of 12.0 nM, 185 kBq of ^{14}C ($NaH^{14}CO_3$, in distilled water), 74 kBq of ^{109}Cd (in 0.1 N HCl) to yield 100 pM, 74 kBq of ^{57}Co (in 0.1 N HCl) to yield 22.8 pM, 268 kBq of ^{51}Cr (in 0.1 N HCl) to yield 1.2 nM, 37 kBq of ^{75}Se (selenite, in distilled water) to yield 1.2 nM, and 74 kBq of ^{65}Zn (in 0.1 N HCl) to yield 60.8 nM. Because we performed metal analysis by measuring sample radioactivity, experimental procedures did not strictly adhere to trace metal clean techniques. Consequently, the concentration of Zn—a ubiquitous contaminant—may have been higher than the background Zn concentration (this was not examined), thereby reducing the specific activity of the ^{65}Zn in the medium. The pH of the seawater was adjusted to 8.0 by adding microliter

quantities of 0.5 N Suprapur NaOH. The amounts of radioisotope added to the algal cultures were chosen to facilitate detection of radioactivity in copepods and fecal pellets following feeding.

After 4–8 d, the cells had undergone several divisions (4–6) and were considered uniformly labeled. Radiolabeled algal cells were collected by either filtration (1-μm filter) or centrifugation (for *R. salina* only, as described above), rinsed with unlabeled filtered seawater, and resuspended into 50 ml of filtered seawater.

The assimilation in copepods of metals bound to inorganic particles was assessed by feeding the animals glass beads (5–10 μm) coated with Fe oxide, prepared according to Decho and Luoma (1994). Fe oxide particles were then labeled with either ^{109}Cd and ^{57}Co or ^{241}Am and ^{65}Zn for 1 d (^{75}Se did not adsorb onto these particles). Radioisotope additions were 74 kBq each of ^{241}Am, ^{109}Cd, ^{57}Co, and ^{65}Zn. The pH of seawater was adjusted to 8.0 as above. The labeled particles were collected by centrifugation and resuspended into 100 ml of filtered seawater. This procedure was repeated twice to remove weakly bound metals.

Feeding experiments—A known amount of algal cells (1–4 ml) from the 50-ml cell concentrates was added to 150 ml of filtered seawater in a 240-ml polypropylene beaker. In food quantity experiments with *Thalassiosira pseudonana,* four cell concentration treatments were prepared: 2×10^3, 7×10^3, 2×10^4, and 10^5 cells ml^{-1} (equivalent to 16, 56, 160, and 800 μg C liter^{-1}). In experiments comparing different algal species, algal cell densities in the feeding suspensions ranged from 1 to 2×10^4 cells ml^{-1}. The cells were allowed to equilibrate for 30 min, after which the fraction associated with the cells (particulate phase) and the cell counts were determined (Fisher et al. 1983*a*). In each experimental group, there were 3 replicate beakers for each treatment, one control beaker without any copepods but with labeled algal cells (to monitor metal desorption from algal particles), and one beaker with the same amount of dissolved radiotracers (after the labeled algal cells were filtered out) to monitor uptake of radioisotopes by the animals from the dissolved phase during the radioactive feeding period.

Copepods were then added to the feeding containers at an approximate density of 500 animals liter^{-1}. After 1 h of feeding on labeled cells in the dark, copepods were rapidly collected with a 160-μm nylon mesh (taking care to retain a small amount of water to avoid desiccation), rinsed with filtered seawater, and their radioactivity assayed. Fecal pellets were collected by a finer nylon mesh (20 or 36 μm, depending on fecal pellet sizes), rinsed, and their radioactivity assayed. The percent of metals associated with the particulate phase was also determined as described above. After copepods were fed, phytoplankton cell densities were determined with concentrated samples by passing 50 ml of each feeding suspension through a 1-μm polycarbonate membrane and resuspending the collected algae into 2 ml of filtered seawater (cell densities of *R. salina* were not measured because it could not be recovered off polycarbonate membrane).

After 1 h of feeding on radioactive phytoplankton and radioactive measurements, the radioactive copepods were allowed to purge their guts of radiolabeled food by feeding on unlabeled food (same food conditions) in 100 ml of 0.2-μm-filtered seawater for 4 h. After this period of gut evacuation, the radioactivity of the copepods and the collected fecal pellets was measured. Assimilation efficiency (AE) is defined here as the amount of radioactivity retained after 4 h of depuration divided by the amount ingested during the 1-h period of radioactive feeding (mass balance method). The amount ingested was calculated as the radioactivity of copepods plus fecal pellets. Mean AEs (in percentages) were calculated with arcsine transformations of the three replicate measurements to meet the assumptions of ANOVA (Sokal and Rohlf 1981). Carbon AE was calculated by either the mass balance method or the ^{14}C:^{51}Cr ratio method (Calow and Fletcher 1972) using the equation

$$\mathrm{AE} = \left[1 - \frac{(^{14}\mathrm{C}/^{51}\mathrm{Cr})_{\mathrm{feces}}}{(^{14}\mathrm{C}/^{51}\mathrm{Cr})_{\mathrm{food}}}\right] \times 100. \qquad (1)$$

$(^{14}\mathrm{C}/^{51}\mathrm{Cr})_{\mathrm{feces}}$ is the ratio of ^{14}C to ^{51}Cr in copepod fecal pellets collected after the 1-h period of radioactive feeding and after the 4-h period of depuration, and $(^{14}\mathrm{C}/^{51}\mathrm{Cr})_{\mathrm{food}}$ is the ratio of ^{14}C to ^{51}Cr in food particles during the radioactive feeding period.

In Fe oxide feeding experiments, labeled particles were added to the feeding containers at a concentration of 10^5 particles ml^{-1}. After the copepods were added, the flasks were sealed and placed on a spinning wheel (2 rpm) for 1 h to keep the particles in constant free fall (Lee and Fisher 1994). Unlabeled *R. salina* cells were added to some of the radiolabeled Fe oxide feeding flasks to stimulate the feeding activity of the copepods. The difference between these two treatments was compared.

Physiological turnover of ingested metals in copepods—The physiological turnover rates of assimilated elements were determined by allowing all copepods to depurate for an additional 17 h under the same food conditions after the initial 4-h period of gut clearance. Physiological turnover rates based on radioisotope loss from 4 to 21 h after the radioactive feeding were then calculated for each treatment.

Releases of elements from decomposing fecal pellets—In experiments with *R. salina, Prorocentrum minimum,* and *Tetraselmis maculata,* fecal pellets produced after the 1-h period of radioactive feeding were collected and their radioactivity was assayed. The fecal pellets were then transferred into replicate vials (10 ml per vial) containing 0.2-μm-filtered seawater and incubated at 15°C. The release of incorporated elements from fecal pellets was measured over a time-course of 61–64 d, following procedures described by Fisher et al. (1991*a*).

Analytical procedures—The radioactivity of γ-emitting isotopes in copepods and fecal pellets was measured with a large well NaI(Tl) gamma detector interfaced with a

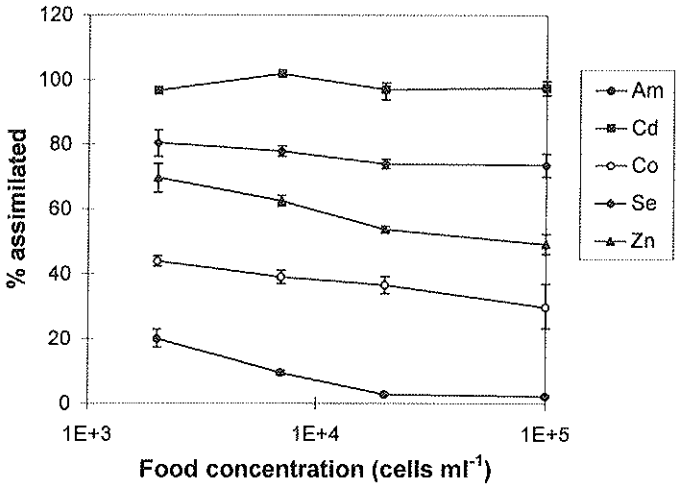

Fig. 1. Assimilation efficiencies of Am, Cd, Co, Se, and Zn in marine copepods fed on different food concentrations of *Thalassiosira pseudonana* (clone 3H). Values shown are means ± SD ($n = 3$).

Table 2. Comparison of C assimilation efficiencies (AE, mean ± SD, $n = 3$) by marine copepods calculated by the mass balance method (amount retained divided by amount ingested) and the $^{14}C:^{51}Cr$ ratio method.

Food	Mass balance AE	$^{14}C:^{51}Cr$ AE	$^{14}C:^{51}Cr$ in labeled food
Thalassiosira pseudonana			
2×10^3 cells ml^{-1}	94.3±0.4	94.4±0.6	12.69
7×10^3 cells ml^{-1}	92.8±0.9	96.6±0.4	12.90
2×10^4 cells ml^{-1}	90.9±1.6	94.9±0.3	10.48
1×10^5 cells ml^{-1}	88.3±1.4	93.1±0.6	10.44
Phaeodactylum tricornutum	80.0±0.4	92.6±0.9	8.76
Chlorella autotrophica	75.9±1.5	74.6±1.6	12.31
Tetraselmis levis	75.5±4.4	80.0±4.9	8.28
Tetraselmis maculata	76.3±5.1	75.6±6.0	11.72
Prorocentrum minimum	70.6±1.8	81.0±1.6	9.87
Rhodomonas salina	72.9±4.6	68.7±5.5	6.70

multichannel analyzer. The radioactivity of water and algal particles was determined by a Pharmacia-Wallac LKB gamma counter equipped with a well-type NaI(Tl) crystal. The two gamma counters were intercalibrated with radioisotope standards. The γ emissions of ^{241}Am were detected at 60 keV, of ^{109}Cd at 88 keV, of ^{57}Co at 122 keV, of ^{51}Cr at 320 keV, of ^{75}Se at 264 keV, and of ^{65}Zn at 1,115 keV. Counting efficiencies for each isotope were measured for both detectors with appropriate standards. ^{14}C activity was determined with an LKB Rack Beta liquid scintillation counter, and the external standards ratio method was used to correct for quenching errors. Counting times in all samples were adjusted so that propagated counting errors were generally <5%.

Results

Assimilation of ingested elements—Element AEs in copepods fed the diatom *T. pseudonana* at cell densities ranging from 2×10^3 to 10^5 cells ml^{-1} are shown in Fig. 1. Food concentration had the greatest effect on the assimilation of ^{241}Am and ^{65}Zn; AEs at food concentrations of 2×10^3 and 7×10^3 cells ml^{-1} were significantly higher ($P < 0.05$, "Student's" t-test) than those at 2×10^4 and 10^5 cells ml^{-1}. For other elements (^{109}Cd, ^{57}Co, and ^{75}Se), there was no statistically significant difference in AE for all food treatments (2×10^3 to 10^5 cells ml^{-1}), suggesting that food quantity had little influence on the assimilation of these elements.

The mass balance method and the $^{14}C:^{51}Cr$ ratio method gave similar C AEs for each food treatment, except for *Phaeodactylum tricornutum* and *P. minimum*, in which AEs were higher when calculated by the $^{14}C:^{51}Cr$ ratio method than by the mass balance method (Table 2). When calculated by the mass balance method, there was a slight decrease in AEs with increasing food concentration, but AEs calculated by the $^{14}C:^{51}Cr$ ratio method were relatively constant throughout the range of food concentrations tested (Table 2). C assimilation from the two diatoms studied (*T. pseudonana* and *P. tricornutum*) was higher than from the other algal species; however, C from all algal sources was assimilated by copepods with a high efficiency (71–95%).

There was little variation in copepod AE for each element (Fig. 2) among the seven algal species tested. Two experiments were conducted for ^{241}Am, ^{75}Se, and ^{65}Zn, and the results were comparable. AEs in copepods fed *Tetraselmis levis* showed the highest deviation from the mean AEs. Because two algal species, *Chlorella autotrophica* and *R. salina*, did not concentrate significant amounts of ^{75}Se, AEs of ^{75}Se from these two species were not determined. Among the five trace elements, ^{109}Cd was assimilated most efficiently (85–100%) (Table 3, Fig. 2). Fecal pellets collected after 1 h of "hot" feeding and 4 h of "cold" depuration accounted for only a minor fraction of metal retained in copepods, confirming that this element was efficiently retained in the animals (Table 3). Copepods also assimilated ^{75}Se and ^{65}Zn with high efficiencies (50–90%). ^{57}Co was assimilated with intermediate efficiencies (25–48%), while the nonessential element ^{241}Am, with AEs < 10% for all algal species, was retained the least.

Metals associated with Fe oxides were generally assimilated with lower efficiencies (~50% less) than metals assimilated from phytoplankton food (Table 4). The only exception was ^{241}Am, which copepods assimilated more (23%) from Fe oxides than from algal food (<10%). The additions of unlabeled algal food (*R. salina*) to the hot Fe oxide feeding suspensions did not significantly affect the AEs of ^{109}Cd and ^{65}Zn, but copepods assimilated less ^{241}Am and ^{57}Co from Fe oxides when the algae were present.

Generally, uptake of radioisotopes from the dissolved phase during the radioactive feeding periods represented

Table 3. Proportion of ingested trace elements associated with copepods (retained), fecal pellets (egested), and the dissolved phase after 4 h of depuration in unlabeled seawater (mean ± SD, $n = 3$); nd—not determined. Proportions were calculated as fractions (%) of the total radioactivity in each component after 1 h of feeding. The fractions retained in the animals were therefore different from the fractions assimilated (*see Figs. 1, 2*).

Fraction	Am	Cd	Co	Se	Zn
Thalassiosira pseudonana (2×10^3 cells ml^{-1})					
Retained	30.0±5.3	97.6±0.9	49.0±3.2	79.0±4.0	78.4±5.0
Egested	50.9±6.9	0.7±0.4	11.8±0.9	2.4±1.4	13.5±2.9
Dissolved	23.8±7.8	1.8±0.5	39.2±2.4	18.5±2.2	6.5±2.4
(7×10^3 cells ml^{-1})					
Retained	23.4±2.5	102.7±1.3	44.0±1.9	79.3±3.2	79.5±3.2
Egested	40.1±1.3	1.1±0.4	6.2±0.2	0.8±0.0	6.0±1.0
Dissolved	36.4±1.2	0±0	49.8±2.0	19.9±3.3	14.5±4.1
(2×10^4 cells ml^{-1})					
Retained	10.8±1.7	97.7±3.0	44.4±3.4	75.8±1.4	78.8±1.4
Egested	41.2±4.9	0.5±0.4	4.2±0.3	0.9±0.5	5.3±2.0
Dissolved	48.0±3.5	2.7±2.1	51.4±3.1	23.2±1.0	15.8±2.0
(1×10^5 cells ml^{-1})					
Retained	11.5±2.6	98.5±2.2	39.4±8.7	72.0±4.0	76.3±3.8
Egested	33.0±1.8	0.4±0.0	3.8±1.1	1.3±0.4	3.9±0.6
Dissolved	55.5±4.7	1.8±1.3	56.8±7.7	26.7±3.7	19.9±3.3
Phaeodactylum tricornutum					
Retained	7.2±1.4	93.5±4.0	38.7±2.2	88.5±1.9	84.9±2.6
Egested	6.9±0.8	0.1±0.1	2.9±1.2	0.6±0.2	0.3±0.3
Dissolved	85.8±2.1	6.4±3.9	58.4±1.4	10.9±1.9	14.8±2.6
Chlorella autotrophica					
Retained	17.8±1.4	95.0±6.5	44.0±8.1	nd	83.8±4.0
Egested	42.6±3.8	0.6±0.5	6.2±2.6	nd	3.0±0.8
Dissolved	39.6±2.5	5.6±5.6	49.8±8.8	nd	13.1±3.8
Tetraselmis levis					
Retained	12.8±0.8	94.1±1.8	53.7±3.8	80.5±1.1	84.0±0.5
Egested	27.1±6.2	0.5±0.0	2.1±0.4	1.7±0.4	3.0±1.0
Dissolved	60.1±5.3	5.4±1.8	44.2±3.8	17.7±1.3	12.9±1.2
Tetraselmis maculata					
Retained	20.8±1.1	92.1±2.8	33.7±4.6	80.7±0.6	88.2±0.6
Egested	27.2±3.1	1.3±0.7	3.7±0.3	0.8±0.0	1.1±0.2
Dissolved	51.9±3.4	6.6±3.4	62.6±4.5	18.5±0.7	10.7±0.3
Prorocentrum minimum					
Retained	9.3±2.8	88.3±2.1	26.6±2.1	73.3±1.0	83.0±1.5
Egested	31.6±4.1	0.9±0.2	2.1±0.2	2.3±0.1	1.3±0.3
Dissolved	59.2±3.0	10.8±2.1	71.2±2.0	24.4±1.0	15.7±1.8
Rhodomonas salina					
Retained	9.4±1.3	99.2±1.8	44.6±1.4	nd	0.8±1.9
Egested	25.5±7.2	0.9±0.4	4.5±1.0	nd	1.9±0.9
Dissolved	65.0±7.0	0.9±0.8	50.9±1.7	nd	17.2±2.6

a negligible fraction of total metal accumulation; the highest contribution was observed for ^{241}Am, with ~5% of the total accumulation coming from the dissolved phase. After 1 h of feeding, from 10 (*C. autotrophica*) to 30% (*R. salina*) of algal cells were ingested by the copepods.

Egestion and regeneration of ingested elements during depuration—The proportion of ingested trace elements in copepods (retained), fecal pellets (egested), and water were determined following the 4-h period of depuration (Table 3). With the exception of ^{241}Am, trace elements in fecal pellets accounted for only a minor fraction of total ingested activities after 4 h of depuration. Significant fractions of Am, Co, Se, and Zn were recovered in the depuration water, including truly dissolved elements and those associated (by adsorption) with unlabeled food particles. ^{109}Cd was assimilated with very high efficiencies in the copepods, and only a small fraction of ingested metal was regenerated into the water during depuration (<10%). For ^{75}Se and ^{65}Zn, the dissolved pools represented ~10–25% of that ingested in copepods, whereas 40–70% of ingested ^{57}Co was regenerated into the dis-

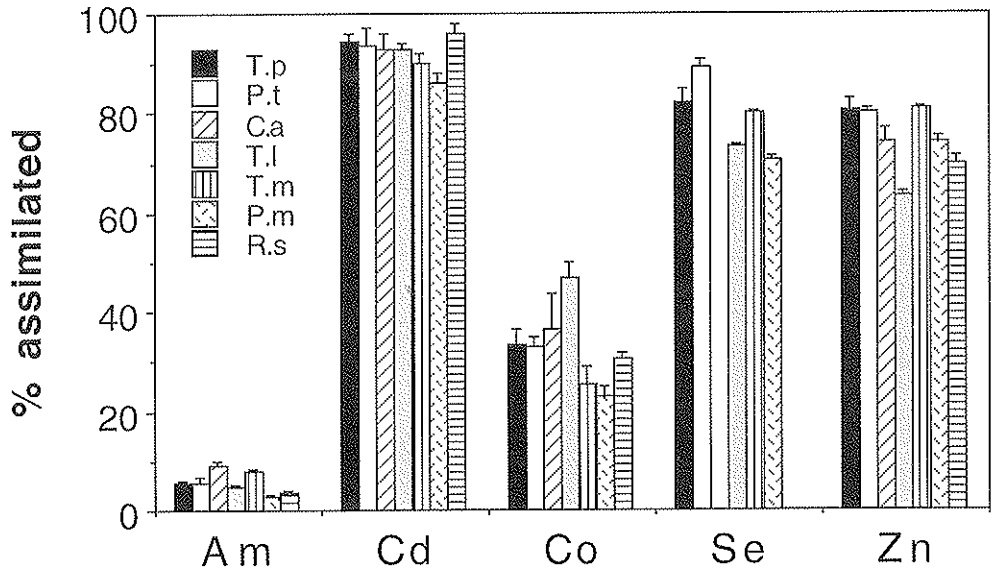

Fig. 2. Assimilation efficiencies of Am, Cd, Co, Se, and Zn by marine copepods fed on different algal diets. Values shown are means ± SD (n = 3). T.p—*Thalassiosira pseudonana*; P.t—*Phaeodactylum tricornutum*; C.a—*Chlorella autotrophica*; T.l—*Tetraselmis levis*; T.m—*Tetraselmis maculata*; P.m—*Prorocentrum minimum*; R.s—*Rhodomonas salina*.

solved pool. There was little evidence that food type affected trace element partitioning into egested or regenerated pools. Food concentration, however, had significant influence on elemental egestion and regeneration such that at higher food concentrations, more ingested metal was regenerated and the proportion associated with fecal pellets declined (Table 3).

Physiological turnover rates of elements in copepods—Physiological turnover rate constants (k) of elements were calculated with the equation

$$k\ (\mathrm{d}^{-1}) = \frac{\ln(A_{21\ \mathrm{h}}) - \ln(A_{4\ \mathrm{h}})}{t}. \qquad (2)$$

$A_{21\ \mathrm{h}}$ is the percent retained after 21 h of depuration, $A_{4\ \mathrm{h}}$ is the percent retained after 4 h of depuration, and t is the time interval (0.71 d). This calculation assumed that copepods completed their digestion of ingested food within 4 h and that any loss of metals afterward was due to physiological turnover (regeneration). Among the five trace elements (Table 5), ^{241}Am and ^{57}Co were regenerated with the highest rates (k = 1.3–2.6 d^{-1}), ^{75}Se and ^{65}Zn were regenerated at intermediate rates (k = 0.3–1.1 d^{-1}), and ^{109}Cd was lost from the grazers most slowly ($k \approx 0.5$ d^{-1}). The calculated physiological turnover rates were 77–93% d^{-1} for ^{241}Am, 31–48% d^{-1} for ^{109}Cd, 78–86% d^{-1} for ^{57}Co, 34–69% d^{-1} for ^{75}Se, and 28–55% d^{-1} for ^{65}Zn. There was no clear trend to suggest that both food concentration and algal species significantly influenced the physiological turnover of the trace elements. However, higher k values for ^{75}Se and ^{65}Zn were noted in copepods fed *T. pseudonana* than in animals fed other species (Table 5).

Elemental releases from decomposing fecal pellets—The release of the five trace elements from decomposing fecal pellets is shown in Fig. 3. The trace element retention curves were best described by the equation (Lee and Fisher 1992*b*)

$$y = 100(t + 1)^{-b}. \qquad (3)$$

y is the percentage of isotope retained in the fecal pellets, t is time (d), and b is the release rate coefficient. Values of b and retention half-times ($t_{r\,½}$, d) for five elements and three algal diets were calculated from linear regressions of log-log-transformed data (Table 6).

These results suggest that of the elements examined, ^{109}Cd was released at the highest rate from the fecal pellets ($t_{r\,½}$ of only ~1 d). ^{241}Am was released at the slowest rate

Table 4. Assimilation efficiencies of metals (mean ± SD, n = 3) from Fe oxide particles by copepods. For a calculation of AE, *see text*. In an accompanying experiment, unlabeled *Rhodomonas salina* cells (5×10^3 ml^{-1}) were added into the hot feeding suspension to stimulate feeding activity of the copepods.

	Am	Cd	Co	Zn
Fe oxides only	22.9±7.6	48.7±4.5	21.5±1.2	33.0±5.7
Fe oxides + unlabeled *R. salina* cells	9.9±3.5	45.7±3.6	14.7±1.3	25.1±8.1

Table 5. Physiological turnover rate constant (d^{-1}) of elements in copepods (mean ± SD, $n = 3$); nd—not determined.

Food	Am	Cd	Co	Se	Zn
Thalassiosira pseudonana					
2×10^3 cells ml^{-1}	1.83±0.17	0.38±0.11	1.76±0.06	1.14±0.25	0.61±0.03
7×10^3 cells ml^{-1}	1.78±0.20	0.54±0.10	1.80±0.08	1.12±0.23	0.59±0.09
2×10^4 cells ml^{-1}	1.97±0.41	0.41±0.13	1.71±0.07	1.12±0.03	0.59±0.07
1×10^5 cells ml^{-1}	1.90±0.45	0.54±0.21	1.65±0.16	1.05±0.07	0.49±0.02
Phaeodactylum tricornutum	1.85±0.17	0.44±0.08	1.64±0.33	0.42±0.04	0.32±0.04
Chlorella autotrophica	2.59±0.60	0.51±0.05	1.30±0.93	nd	0.39±0.11
Tetraselmis levis	2.36±0.22	0.65±0.11	1.86±0.11	0.52±0.02	0.33±0.02
Tetraselmis maculata	nd	0.44±0.03	1.77±0.05	nd	nd
Prorocentrum minimum	1.75±0.85	0.42±0.02	2.08±0.07	0.78±0.00	0.42±0.02
Rhodomonas salina	2.36±0.24	0.41±0.09	1.57±0.05	nd	0.34±0.10
Fe oxides	2.62±0.09	0.42±0.06	1.52±0.24	nd	0.70±0.29
Fe oxides + *R. salina*	1.72±0.24	0.44±0.05	1.98±0.28	nd	0.81±0.15

($t_{r\,½}$ of ~10 d). Retention half-times of other elements (^{57}Co, ^{75}Se, and ^{65}Zn) ranged from 1.5 to 3.7 d. Food species had no consistent effect on elemental release rates from fecal pellets.

Discussion

Elemental assimilation—There were notable differences in cell size, morphology, and wall structure among the seven algal species examined. Nevertheless, AEs of each element were similar regardless of the algal species (with or without cell wall) or quantity of ingested phytoplankton food, demonstrating that different algal species and food quantity have little effect on element assimilation in copepods. The range of food concentrations used (16–800 μg C $liter^{-1}$) is representative of coastal waters, including bloom and nonbloom conditions. There were some exceptions to this generalization, as noted above.

Table 6. Release rate coefficients (b) and biological half-times ($t_{r\,½}$, in d) of elements in copepod fecal pellets (mean ± SD, $n = 3$). Values of b and $t_{r\,½}$ were calculated as described in the text.

Element	Food*	b	$t_{r\,½}$	r^2
Am	*P. minimum*	0.273±0.006	11.7±0.7	0.973
	T. maculata	0.279±0.008	11.0±0.9	0.958
	R. salina	0.363±0.019	5.8±0.6	0.934
Cd	*P. minimum*	1.197±0.081	0.8±0.1	0.907
	T. maculata	1.064±0.217	2.0±0.2	0.856
	R. salina	1.074±0.082	0.9±0.1	0.884
Co	*P. minimum*	0.647±0.054	1.9±0.2	0.987
	T. maculata	0.748±0.051	1.5±0.2	0.994
	R. salina	0.517±0.042	2.8±0.5	0.939
Se	*P. minimum*	0.527±0.016	2.7±0.2	0.973
	T. maculata	0.449±0.022	3.7±0.1	0.935
Zn	*P. minimum*	0.660±0.008	1.9±0.0	0.981
	T. maculata	0.719±0.013	1.6±0.0	0.907
	R. salina	0.663±0.019	1.8±0.2	0.965

* *Prorocentrum minimum, Tetraselmis maculata,* and *Rhodomonas salina.*

Conover (1966) also showed that C assimilation was not related to the amount of food (*Thalassiosira weissflogii*) offered nor to the amount ingested by copepods over a cell concentration range of $1–17\times10^3$ cells ml^{-1} (or 0.14–2.4 mg C $liter^{-1}$). Within this concentration range, assimilation was relatively constant at ~70%. Conover (1966) also measured the assimilation of C by copepods fed on different food species. Generally, there was a negative correlation between AE and ash content of food particles, and C in flagellates, with a low ash content, was assimilated with the greatest efficiency. However, there is little variation in carbon AE when copepods feed on different natural assemblages of phytoplankton (Butler et al. 1969; Taguchi and Ishii 1972). Abou Debs (1984) observed no difference in C assimilation by *Temora stylifera* feeding on the haptophyte *Hymenomonas elongata* and the diatom *P. tricornutum* and also found no appreciable effect of food quantity on assimilation.

Gut transit time is important for determining food assimilation in animals (Sibly and Calow 1989) and has been one of the major parameters in modeling optimal digestion in suspension feeders such as bivalves (Willows 1992). A longer gut residence time often leads to more efficient digestion and assimilation of the ingested food materials because digestive enzymes have a longer time to act upon them. Gut passage time in marine copepods seems to be species-specific and varies with different measuring techniques, ranging from 30 min to 3 h as measured by gut fluorescence or radiotracer techniques (Marshall and Orr 1972; Dagg and Grill 1980). Dam and Peterson (1988) summarized the general relationship between gut clearance rate and temperature as $k = 0.0117 + [0.001794][T]$, where k is gut clearance rate and T is temperature. With this equation, the calculated k for copepods under our experimental condition (15°C) is 0.038 min^{-1}, and gut transit time is ~26 min. This is fully consistent with our observations that the gut transit time in *A. tonsa* was generally <30 min because fecal pellets collected after *A. tonsa* fed for 30 min on ^{241}Am-labeled

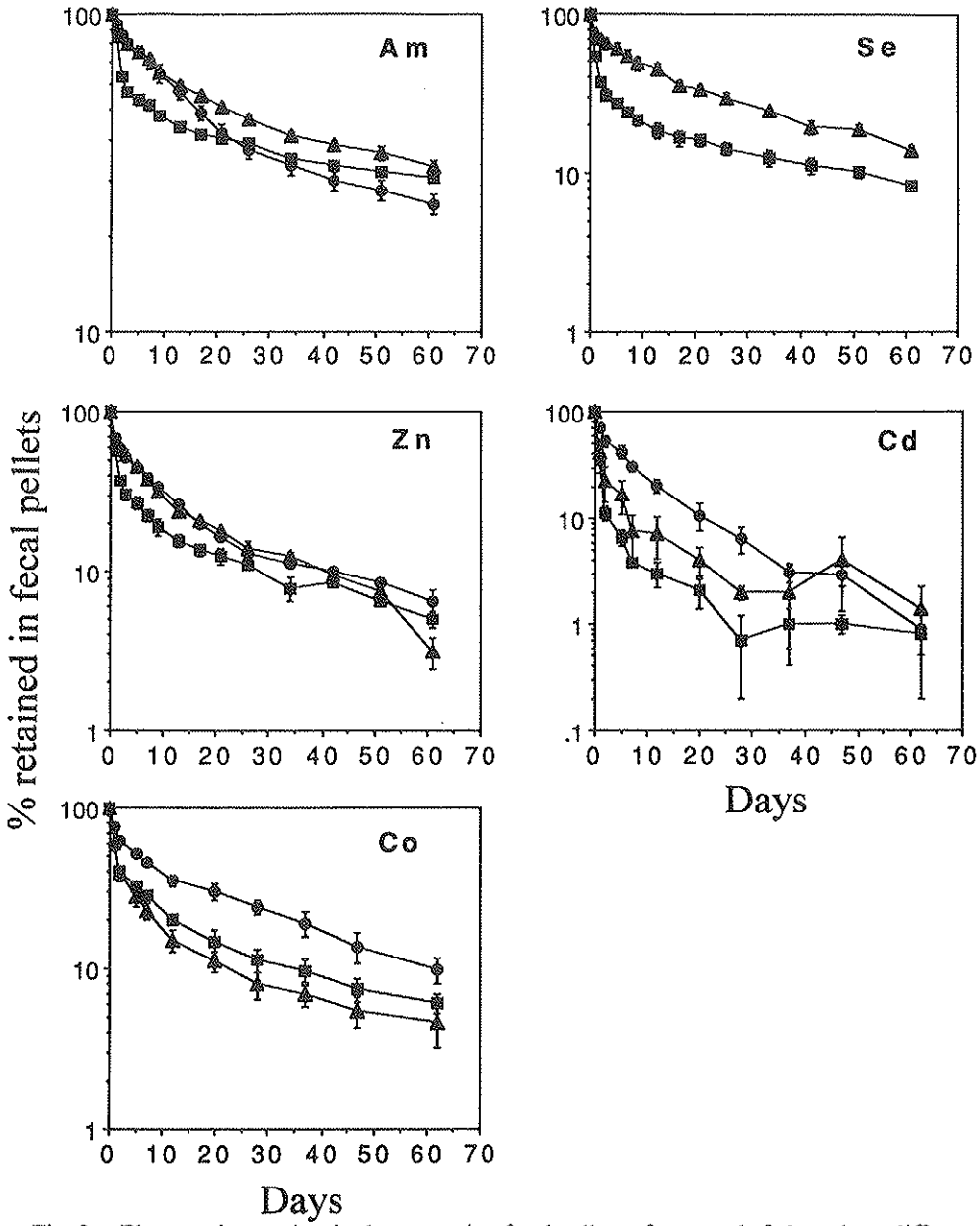

Fig. 3. Elemental retention in decomposing fecal pellets of copepods fed on three different algal diets. Values shown are means ± SD (n = 3). *Prorocentrum minimum*—■; *Tetraselmis maculata*—▲; *Rhodomonas salina*—●.

algae contained significant amounts of radioactivity (this element can be used as an inert tracer of food passage in copepods, Fisher and Reinfelder 1991). The gut transit time seems to be independent of the feeding conditions of copepods (Ellis and Small 1989). Dam and Peterson (1988) also suggested that >75% of the variation of copepod gut transit time could be explained by differences in temperature, while food species and concentration had little effect on gut passage.

Reinfelder and Fisher (1991) suggested the existence of a "liquid" digestion-assimilation strategy for marine copepods, by which they fully assimilate the cytoplasm of prey cells. This strategy can represent one type of optimal feeding by which assimilation is conserved, presumably because of the higher energetic costs associated with increasing the assimilation of ingested particles (18–28% of total energy associated with feeding, Kiørboe et al. 1985). In the short gut transit time typical of copepods, assimilation may thus play a small role in optimizing energy and nutrient gain during feeding. Instead, copepods may respond to changes in feeding conditions with changes in ingestion rates and food selectivity (Peters and Downing 1984; Paffenhöfer and Van Sant 1985). The energetic costs of ingestion and food transport within the gut represent a negligible percentage of total energy expenditure in copepods (~1%, Kiørboe et al. 1985).

The accumulation of metals in copepods from ingested particles is determined by metal AE, metal concentration

and partitioning in the food particles, copepod ingestion rates, and the efflux rate of the metals from the copepods. Although AEs of all metals were relatively constant for a variety of algal species, metal accumulation may show large variability due to variations in metal concentration in food particles and copepod feeding activity. Pronounced differences in copepod feeding rates were noted for different algal diets, with the chlorophyte *C. autotrophica* ingested the least and the cryptophyte *R. salina* ingested the most. Because *C. autotrophica* and *R. salina* did not appreciably concentrate Se from ambient seawater, Se influx into copepods from these foods was lower than for other algal species.

Digestive processing of Fe oxide particles may be different from that of algal cells. The AE from these particles was consistently lower (~50% less for Cd, Co, and Zn) than from algal cells. The assimilation of metals from Fe oxide particles may depend entirely on metal desorption in the copepod gut, which has a pH of ~5 (Dall and Moriarty 1983). It is also possible that Fe released from the oxides in the acidic gut could compete with other metals for uptake across the gut lining, which would lower the AEs for the other metals.

Carbon AEs in copepods in this study were comparable to literature values (~70%, Conover 1978); however the C assimilation measured at the highest cell density of *T. pseudonana* (88%) was comparable to that measured by Reinfelder and Fisher (1991) under similar experimental conditions. Overall, AEs of carbon from diatoms were somewhat greater than AEs from other algae, indicating that more of the C in the nondiatoms was refractory (perhaps as components of cell walls).

Although AEs for Am and Co were comparable to previous measurements, AEs of Cd and Zn were higher (70–90%) and of Se somewhat lower (70–80%) than previous measurements (30–60% for Cd and Zn and 97% for Se) (Reinfelder and Fisher 1991; Fisher et al. 1991*b*). In our study, we used a shorter radioactive feeding period (1 h) and depurated the copepods for 4 h to avoid metal recycling. The cytoplasmic distribution of elements, and thus their AEs in copepods, varied with the physiological state of the algal cells (Reinfelder and Fisher 1991, 1994). All algal cells used in this study were in log phase growth. The higher AE observed here for Cd is difficult to explain.

Nolan et al. (1992) argued that only the organic form of Co (cobalamine) is bioavailable to marine diatoms, but other studies indicate that under Zn-limited conditions, ionic Co may substitute for Zn (Price and Morel 1990). The inorganic Co we presented to the algae primarily binds to cell surfaces (>70%, Reinfelder and Fisher 1991, 1994), which may explain its low assimilation in copepods. By contrast, Se and Zn penetrate more effectively into algal cytoplasm and are assimilated with higher efficiencies (Reinfelder and Fisher 1991, 1994). Am, which is almost completely bound to algal cell surfaces (Fisher et al. 1983*c*, 1991*b*; Reinfelder and Fisher 1991), consistently shows the least assimilation in grazers.

Metal regeneration by marine copepods—This study provides the first quantitative measure of element regeneration by marine copepods and suggests that regeneration can be an important route by which elements are released from copepods into the dissolved phase. Depending on the element, the turnover rate constants under these experimental conditions ranged from 0.3–2.6 d^{-1}, indicating that regeneration can recycle elements into the dissolved phase very efficiently. Because the copepods were not uniformly radiolabeled, these calculated physiological turnover rates are upper estimates. The importance of element remineralization during zooplankton grazing has recently been examined by Hutchins and Bruland (1994) and Lee and Fisher (1994). Hutchins and Bruland fed the zooplankton grazers with labeled planktonic prey for 9–10 h and measured the distributions associated with the dissolved fraction, fecal pellets, and grazers over time. At the end of feeding, metal concentrations (Fe, Mn, and Zn) in dissolved phases were 3–7 times higher in bottles with grazers than in control bottles without grazers. Lee and Fisher (1994) demonstrated that the release of trace metals from radiolabeled diatoms into the dissolved phase over 40 h was enhanced 5–15% by zooplankton grazing.

Because these earlier studies incubated copepods for long periods, two mechanisms may have contributed to the observed increase in dissolved metal pools. First, there may have been significant remineralization by marine copepods, either from physiological turnover of metals after assimilation, from loss into the dissolved phase, from egested fecal pellets, or from sloppy feeding behavior of copepods. Second, a decrease in prey cell density due to grazing may have resulted in a change in metal particle-water partitioning (leading to an increase in dissolved metal pools) if the metal concentration factor remained constant. In our 1-h feeding experiments, we observed that compared to controls without copepods, the fraction of elements (especially Am, Co, Se, and Zn) in the dissolved phase increased significantly, primarily due to a decline in food concentration. The calculated concentration factors in the algal cells remained constant throughout this feeding period. Thus, change in particle concentration needs to be considered in long-term incubation experiments.

The physiological turnover of assimilated elements seems to be independent of food species or the amount of food ingested. Among the five trace elements studied here, only Zn was regenerated at a higher rate (k = 0.61 d^{-1}) when food concentrations were low than it was when food concentrations were high (k constant = 0.49 d^{-1}). Our calculated physiological turnover rates for Se, Zn, and Cd were comparable to those of major nutrient elements in copepods. Conover (1961) measured a P turnover rate of 10% d^{-1} for the copepod *Calanus finmarchicus,* and Corner et al. (1972) found a P turnover rate of 41% d^{-1} by *Calanus helgolandicus.* A daily P turnover rate of 35–60% was observed for *Daphnia* (Lehman 1980). The similarity in the regeneration rates by marine zooplankton of major nutrient elements and of Se, Zn, and Cd is consistent with and may contribute to oceanographic observations in which vertical profiles of these elements (e.g. Cd vs. P, Zn vs. Si) are also similar (Broecker and Peng 1982).

After 4 h of depuration, the percent of each trace ele-

ment in the dissolved phase generally increased with increasing food concentrations. The dissolved term was calculated as the sum of truly dissolved elements and those associated with food particles because the latter could only originate from the dissolved source (these particles were introduced into the cultures without radiolabel). For example, the dissolved fraction of Zn increased from 6% at 2×10^3 cells ml^{-1} to 20% at 10^5 cells ml^{-1}. This relationship of trace element regeneration with food concentration is consistent with findings from other studies that have shown that regeneration rates of N and P increase with an increase in food concentration (Butler et al. 1970; Kiørboe et al. 1985). Recently, Hutchins et al. (1995) demonstrated the importance of Fe regeneration by marine copepods. At the end of 4 h of depuration in unlabeled seawater, ~50% of ingested Fe was partitioned into the dissolved phase. The fractions of Fe in copepods (retained) and fecal pellets (egested) were each ~25%.

Given the high density of grazers in the containers, it is possible that the radioactivity detected in the dissolved pool after 4 h of depuration (Table 3) was enhanced by coprorhexy (breaking of fecal pellets) of copepods (Noji et al. 1991). This process may facilitate the release of elements into the dissolved phase, although this has not been carefully studied. Additionally, rapid desorption of radioisotopes from radiolabeled fecal pellets into the dissolved phase may have contributed to the radioactivity in the dissolved phase (Fisher et al. 1991*b*; Lee and Fisher 1992*b*). The relative contribution of each source was not measured in this study.

Several potential errors may stem from our calculation of element AEs by the mass balance method. Element excretion was not considered either during the 1-h period of hot feeding or during the 4-h period of depuration. Consequently, AE may be underestimated. If we assume that elements are regenerated at a rate of 50% d^{-1} (or 8.3% for 4 h), experimental errors due to the lack of an excretion term should be relatively small (<8.3%) and should equal $[8.3\%/(1 + A_{fp}/A_{cop})]$, where A_{fp} and A_{cop} are radioactivity of fecal pellet and copepods measured after 1 h of radioactive feeding. Because >70% of Am ingested by copepods was lost in fecal pellets during the 1-h period of radioactive feeding, this error would be much smaller (i.e. only 2.5%). It is also possible that fecal pellets could scavenge metals from the dissolved phase. The loss of metals from egested fecal pellets into the dissolved phase could be another experimental artifact (leading to an overestimation of AE), but this should be relatively small, because retention half-times for these elements are generally several days (Table 6, Lee and Fisher 1992*b*). Furthermore, copepod fecal pellets are encapsulated by a chitinous, peritrophic membrane that is relatively impermeable and may hinder the diffusion of solute from the particles, at least during the initial few hours. Lee and Fisher (1992*b*) found that >90% of organic C was retained at least 1 d, after which it leached gradually into the surrounding water.

Metal release from decomposing fecal pellets—The species of phytoplankton food seems to have little influence on metal release from decomposing fecal pellets, consistent with observations by Lee and Fisher (1992*b*) who fed copepods two algal species, *T. pseudonana* and *Isochrysis galbana*. Release rate coefficients (*b* values) for Zn measured in this study were comparable to those measured by Lee and Fisher (1992*b*), while our *b* values for Am were generally higher. Release rates of Cd and Se from copepod fecal pellets have not been previously reported. We found that Cd was rapidly lost to the dissolved phase and had a $t_{r\,1/2}$ in fecal pellets of only ~1 d. This element is also released rapidly from copepod carcasses and phytoplankton debris and has a retention half-time in the particles of 0.4–6 d (Lee and Fisher 1992*a*, 1994; Reinfelder et al. 1993). The higher release rates of Cd, Se, and Zn may be due to their association with organic ligands in algal cells, resulting in a higher partitioning into the rapidly exchanging pool in fecal pellets. These elements are released at rates comparable to rates of C (Lee and Fisher 1993). Lee and Fisher (1994) also presented evidence that bacterial decomposition and leaching influences the release of Cd, Se, and Zn from fecal pellets, whereas desorption and fragmentation are more important for the release of nonessential elements, such as Am and possibly Co.

Biogeochemical implications—The residence time of trace metals in surface waters can be profoundly affected by biological processes, such as metal adsorption to and desorption from biogenic particles or metal trophic transfer in planktonic food webs (Fisher and Reinfelder 1995). Metals follow one of three distinct pathways during grazing: they are assimilated, regenerated, or egested in fecal material. Assimilated metals are retained in the grazers and can be transferred to higher trophic levels or regenerated by physiological turnover. Regeneration of metals by these grazers can contribute significantly to dissolved pools. Unassimilated elements contained in fecal pellets are either exported from surface waters, re-ingested by detritivores, or released into the water through leaching and particle decomposition.

Among the five trace elements studied here, the particle-reactive element Am should be rapidly exported into deep waters because of its low assimilation by marine zooplankton (Fisher et al. 1983*b*; Reinfelder and Fisher 1991) and greater $t_{r\,1/2}$ in fecal pellets (10–50 d; Fisher et al. 1983*b*, 1991*a*; Lee and Fisher 1992*b*; this study), which sink at rates on the order of 100 m d^{-1} (Angel 1984). These removal and transport mechanisms may explain the brief surface residence time of this (and similar) element(s) in the ocean (Cherry et al. 1983). By contrast, the surface residence time of Cd, Se, and Zn can be significantly enhanced by zooplankton grazing. These elements are highly assimilated by zooplankton (Sick and Baptist 1979; Reinfelder and Fisher 1991, 1994), and once incorporated into the fecal pellets, they are rapidly released into the dissolved phase.

Moreover, regeneration rates for these elements are very similar to major nutrient elements. All these processes can increase metal recycling in surface waters and are consistent with oceanic observations on the geochemical behavior of these elements (Whitfield and Turner 1987). Nevertheless, the chemical speciation of regenerated met-

als and their bioavailability to marine phytoplankton are largely unknown and should be examined to determine their ultimate fate. The influence of microzooplankton on the cycling of trace elements is also largely unstudied, yet these organisms are frequently dominant grazers in pelagic surface waters and may significantly affect geochemical cycling in oceanic regions.

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