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Effects of sinking and zooplankton grazing on the release of elements from planktonic debris

Byeong-Gweon Lee*, Nicholas S. Fisher**

Marine Sciences Research Center, State University of New York, Stony Brook, New York 11794-5000, USA

ABSTRACT: Laboratory radiotracer experiments compared the effects of zooplankton grazing and microbial decomposition on the release of organic C and Ag, Cd, Co, Pb and Po from phytoplankton cells. After 40 h incubation of diatoms with copepods + microorganisms, 70% of the diatom cellular C was remineralized to CO_2 (40%), assimilated in copepod tissue (20%), excreted in fecal pellets (5%) or released as DOC (5%); microorganisms alone removed half this amount of diatom C. Copepod grazing enhanced the conversion of Ag from diatom cells to other forms (particulate and dissolved) by 27%, Po by 25%, Pb by 20%, Cd by 13% and Co by 10% over those cells incubated with only microorganisms; zooplankton grazing increased by 5 to 15% the release of elements from diatom cells into the dissolved phase. Decomposing copepod fecal pellets, held free-falling on a spinning wheel, lost about 20% more C, Ag, Co and Pb than did undisturbed fecal pellets, while no appreciable difference was observed for release of metals from copepod carcasses treated similarly. The results suggest that retention of an element contained primarily in the cytoplasm of phytoplankton cells (such as C or Cd) is largely governed by microbial activity and leaching. Microbial activity has a smaller effect on particle-reactive elements (Ag and Pb) bound to structural components of cells; zooplankton are effective in removing these unassimilable elements by grazing and packaging them into rapidly sinking fecal pellets.

KEY WORDS: Cadmium · Carbon · Cobalt · Grazing · Lead · Phytoplankton · Remineralization · Silver · Sinking · Zooplankton

INTRODUCTION

Zooplankton can recycle elements in surface waters by assimilating them and can enhance the rate of removal of unassimilated elements by packaging them into rapidly sinking fecal pellets (Small & Fowler 1973, Reinfelder & Fisher 1991). The efficiency of element assimilation from ingested phytoplankton in copepods and molluscan larvae correlates with the fraction of elements associated with the cytoplasm of the ingested phytoplankton cells (Reinfelder & Fisher 1991, 1994). Elements which are bound to particle surfaces are poorly assimilated in herbivores and are enriched in zooplankton fecal matter (Fowler 1977, Fisher et al. 1991a).

While there is a growing literature on the effects of microorganisms on the release of trace elements from phytoplankton and other planktonic debris (Lee & Fisher 1992a, 1993a), there is relatively little information available on the impact of zooplankton grazing on the release of trace elements from biogenic particles into the dissolved phase, and much of our knowledge is indirectly surmised from data on assimilation efficiency and concentrations of elements in fecal matter (e.g. Fowler 1977, Reinfelder & Fisher 1991). Zooplankton grazing activities can enhance loss of organic material to solution by leakage of dissolved organic material (DOM) during ingestion, digestion and defecation (Jumars et al. 1989, Banse 1990). Further, large particles such as fecal pellets can be mechanically fragmented into smaller ones by zooplankton with only a small proportion of them being ingested (Lampitt et al. 1990, Noji et al. 1991). Intracellular Fe in phytoplankton cells can be recycled among various size classes of phytoplankton and grazing may be an

*Present address: Water Resources Division, U.S. Geological Survey, 345 Middlefield Road, Menlo Park, California 94025, USA

**Addressee for correspondence

important mechanism for its release from cells (Hutchins et al. 1993).

Many laboratory-derived data on the decomposition of biogenic particles and the release of associated trace elements were based on incubations in which particles are settled on the bottom of experimental vessels. As a consequence, diffusion of released materials could be hampered due to a build-up of concentration gradients surrounding the decomposing particles. To avoid this artifact, spinning wheels have been employed to create experimental situations to simulate the free-falling of particles (Jacobsen & Azam 1984, Lampitt et al. 1990). However, no comparisons between incubation methods have yet evaluated the effects of fluid dynamics on the release of elements from decomposing biogenic particles.

This study therefore assessed the impacts of zooplankton grazing and sinking on the release of elements from planktonic debris. It is essentially a continuation of a series of studies which describe the release of carbon and metals from planktonic debris under different conditions (Lee & Fisher 1992a, b, 1993a, b). None of the previous studies assessed the impact of zooplankton grazing or of sinking on metal release rates from planktonic debris, although this information is essential to understanding and predicting the roles of sinking debris on metal flux in oceanic water columns.

Laboratory radiotracer experiments were conducted to: (1) determine the relative role of microorganisms and mesozooplankton on the release rates of C and 5 metals (Ag, Cd, Co, Pb and Po) from decomposing phytoplankton cells; and (2) compare the rates of elemental release from particles incubated on a spinning wheel apparatus (i.e. free-falling particles) and incubated without sinking. These metals were chosen to allow for comparisons between Class A and Class B metals (Nieboer & Richardson 1980), between metals with differing affinities for plankton (Fisher 1986), and between metals with different retention times in planktonic debris (Fisher et al. 1991b, Lee & Fisher 1993a). Of the metals studied, only Co (as Co-cobalamine) is essential, although minor amounts of Co and Cd may substitute for Zn in some Zn-limited diatoms (Price & Morel 1990). Among these metals, Co and Pb display scavenging type profiles in the oceanic water column, and Ag and Cd display nutrient type vertical profiles (Bruland 1983). ^{210}Po has been shown to enter into biological cycles and is highly enriched in marine organisms relative to its radioactive grandparent ^{210}Pb (Shannon et al. 1970, Heyraud & Cherry 1979), being especially enriched in the hepatopancreas of invertebrates (Cherry et al. 1983). Pb and Po display the highest concentration factors in marine phytoplankton, Cd and Co the lowest (Fisher 1986).

MATERIALS AND METHODS

Radioactive diatoms. To produce radioactive diatom cells, *Thalassiosira pseudonana* (Clone 3H) was inoculated into two 1 l Erlenmeyer flasks each containing 500 ml of sterile-filtered seawater (0.2 µm) enriched with f/2 nutrients (Guillard & Ryther 1962) minus trace metals and EDTA. The initial cell density was 5×10^4 cells ml^{-1}. One of the flasks received 19 kBq ^{110m}Ag ($t_{1/2}$ = 250 d, from Amersham) in 93 µl of 0.1 N HNO_3, 55 kBq ^{109}Cd ($t_{1/2}$ = 462 d, from Amersham) in 25 µl of 0.1 N HCl, 55 kBq ^{57}Co ($t_{1/2}$ = 271 d, from Amersham) in 56 µl of 0.1 N HCl, and 26 kBq of ^{210}Pb (^{210}Po) ($t_{1/2}$ = 22.3 yr, from Isotope Products) in 20 µl of 3 N HNO_3. Total metal additions of the added isotopes were 574 ng Ag (5.2 nM), 2.9 ng Cd (26 pM), 197 ng Co (3.5 nM), and 9.2 ng Pb (44 pM). Just prior to isotope additions, microliter quantities of 0.5 N Suprapur NaOH (from Thomas Scientific) were added so that the pH of the seawater was 8.07 after the addition of the isotopes (dissolved in dilute Ultrex acid, from Thomas Scientific). The other flask received 74 kBq of ^{14}C (as $NaH^{14}CO_3$ in distilled water, pH 8.4).

Both cultures were maintained on a 14 h light : 10 h dark cycle at 18 ± 1°C for 4 d. The cultures were shaken daily and two 10 ml and four 1 ml samples were removed from each culture to determine the radioactivity associated with cells and cell density, as in Fisher et al. (1983a). The cell density after the 4 d incubation ranged from 1.3 to 1.5×10^6 cells ml^{-1} (about 1.5 divisions d^{-1}).

Zooplankton grazing experiment. Adult calanoid copepods *Acartia tonsa* Dana and *Temora longicornis* (Müller) were collected from Stony Brook Harbor, Long Island, New York, USA, using a 63 µm plankton net 3 d before the feeding experiments. The copepods were acclimated at low light in 35‰ glass-fiber filtered (GF/C, 1.2 µm nominal pore size) surface seawater collected in the Atlantic 8 km off Southampton, Long Island; they were held at 18 ± 1°C and were fed unlabeled *Thalassiosira pseudonana* during acclimation. Some of the radiolabeled diatoms in each flask were resuspended into two 2 l flasks each containing 1.5 l of sterile-filtered seawater enriched with microorganisms; the diatom cell density was adjusted to 1.0×10^5 cells ml^{-1} (2.2 mg dry wt l^{-1}). (The unused radiolabeled cells were used for an additional experiment, described below.) The microorganisms used for enrichment were prepared by resuspending organisms collected on 0.2 µm Nuclepore filters after filtering 3 l of 3 µm filtered seawater collected 2 h before the experiment from Stony Brook Harbor (water temperature 10°C). The microorganisms were not characterized, but the abundance of microorganisms added to the experimental water should have been identical to that

in Stony Brook Harbor water (i.e. microorganisms were resuspended out of 3 l of harbor water into 3 l of filtered water).

Then, 300 ml of this medium (diatom cells + microorganisms) from each flask was transferred into each of five 500 ml acid-washed flasks. There were 2 pools of radiolabeled cells, one pool being labeled with ^{14}C, the other with ^{110m}Ag, ^{109}Cd, ^{57}Co and ^{210}Pb (^{210}Po). Twenty adult copepods (10 *Acartia tonsa* + 10 *Temora longicornis*) were transferred to each of 2 replicate flasks from 2 groups of 5 flasks (replicates of either ^{14}C or ^{110m}Ag- ^{109}Cd- ^{57}Co- ^{210}Pb [^{210}Po]-labeled samples). One flask from each group was treated with 24 mM formaldehyde (formalin). The remaining 2 flasks from each group contained only diatom cells and microorganisms.

After 40 h incubation at 18°C in the dark, the radioactivities of ^{110m}Ag- ^{109}Cd- ^{57}Co- ^{210}Pb [^{210}Po]-labeled samples were partitioned into several pools. The radioactivity of samples incubated with microorganisms or with microorganisms + formalin was divided into particulate (>0.2 µm, containing algal cells and cellular debris) and dissolved fractions (Fisher et al. 1983a). The radioactivities of samples incubated with copepods were partitioned into fecal pellets, animals, cells and cellular debris, and dissolved fractions (Fisher et al. 1983b). The copepods were transferred out of the flasks using a 210 µm nylon mesh to another vessel containing unlabeled seawater and fed with unlabeled diatom cells for 2 h to evacuate unassimilated radioactive food from the gut (gut transit times in these animals at these temperatures are <30 min; Reinfelder & Fisher 1991). After 2 h, the animals were transferred to counting tubes for radioactivity counting and the fecal pellets produced during this period were combined with the ones collected from the original incubation flask. The fecal pellets were collected on a 20 µm nylon mesh after animals were separated from the feeding media. After both fecal pellets and animals were removed from the original feeding media, the radioactivities in the particulate and dissolved fractions were determined as with the cultures without animals. The radioactivity of ^{14}C-labeled samples was partitioned using the same procedure as ^{110m}Ag- ^{109}Cd- ^{57}Co- ^{210}Pb [^{210}Po]-labeled samples, except that the activities remaining in solution were further divided into remineralized CO_2 and dissolved organic carbon (DOC) (Lee & Fisher 1992a).

Spinning wheel experiment. Experiments compared elemental release rates from zooplankton debris, which was constantly sinking, with rates from debris settled on the bottom of incubation vessels. Radiolabeled copepod fecal pellets and carcasses were collected as above from about 400 copepods (mixtures of *Acartia tonsa* and *Temora longicornis*) after feeding continuously on radiolabeled diatoms for 1 wk; animals were killed by exposure to air for about 5 min after being caught on a mesh (Lee & Fisher 1992b).

One hundred fecal pellets or 20 carcasses were transferred immediately after collection to 2 batches of 6 acid-washed, 50 ml snap-top plastic vials containing the seawater enriched with microorganisms. Two replicate samples for each type of particle (carcasses and fecal pellets, each labeled with either ^{14}C or ^{110m}Ag- ^{109}Cd- ^{57}Co- ^{210}Pb [^{210}Po]) were incubated at 18°C or 2°C in the dark without any disturbance except at sampling time. The remaining 2 vials from each type of particle were placed on a spinning wheel (2 rpm) at 18°C in the dark. For 30 d the radioactivity retained on the particles was periodically measured, as described in Lee & Fisher (1992b). Suspended particles held on the spinning wheel were constantly 'sinking' in their water. No attempts were made to view bacterial colonization of the debris with electron microscopy.

Analytical procedures. The beta activity of ^{14}C-containing samples was determined with an LKB liquid scintillation counter with external standards ratio for quench correction. The radioactivity of ^{110m}Ag, ^{109}Cd, ^{57}Co and ^{210}Pb in the copepod fecal pellets and carcasses contained in the vials was determined with a large-capacity well-type NaI(Tl) gamma detector interfaced with a multichannel analyzer. The radioactivity of samples in a small counting tube was determined in a Pharmacia-Wallac LKB CompuGamma equipped with a well-type NaI(Tl) crystal. The photon emissions of ^{110m}Ag were detected at 658 keV, of ^{109}Cd at 88 keV, of ^{57}Co at 122 keV and of ^{210}Pb 46 keV. The large-capacity well-type detector was calibrated for its energy and counting efficiency with appropriate standards for the geometries of each vessel used. The radioactivity of alpha-emitting ^{210}Po samples was measured with a standard technique involving acid digestion of samples and plating on silver discs prior to alpha counting (Flynn 1968). Counting times of all samples were adjusted so that propagated counting errors were <5%.

RESULTS

Zooplankton grazing experiment

The partitioning of ^{14}C in various pools after 40 h incubation of ^{14}C-labeled *Thalassiosira pseudonana* cells with microorganisms, with microorganisms + copepods, or with microorganisms + formalin, is shown in Fig. 1; replication of results was excellent. The cells incubated for 40 h with microorganisms lost 33% of their carbon and the largest fraction of the released

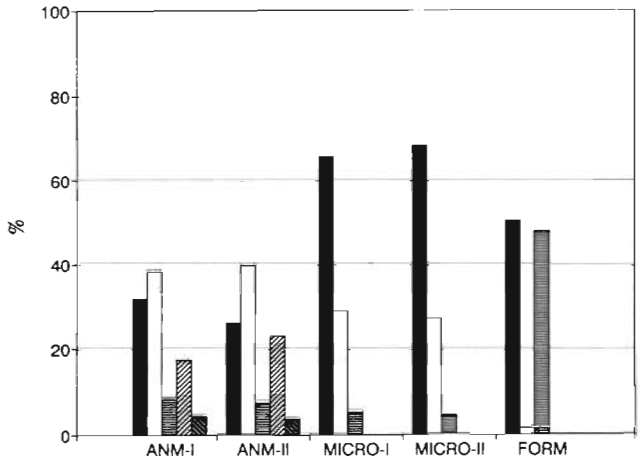

Fig. 1. *Thalassiosira pseudonana, Temora longicornis, Acartia tonsa*. Partitioning of total ^{14}C activity in replicate experiments (I and II) among diatom cells and debris (■), $^{14}CO_2$ (□), $DO^{14}C$ (▩), animal (▨), and fecal pellet (▧) fractions after 40 h incubations of the diatom with either copepods + microorganisms (ANM-I and ANM-II), microorganisms (MICRO-I and MICRO-II), or microorganisms + formalin (FORM). No replicate was used for the formalin treatment

carbon (28% of total carbon) was remineralized to CO_2. After 40 h incubation of diatoms with copepods + microorganisms, 39% of the cellular carbon was remineralized to CO_2, 20% was assimilated in the animals, 28% was retained by the cells and debris, and <10% was in fecal pellets or dissolved fractions. The cells treated with formalin leached half of their cellular carbon as DOC (Fig. 1).

The partitioning of ^{110m}Ag, ^{14}C, ^{109}Cd, ^{57}Co, ^{210}Pb and ^{210}Po into activity retained in animals, fecal pellets, cell debris, and dissolved fractions from the decomposing cells incubated with copepods is given in Fig. 2. The ^{14}C activity in the dissolved fraction is the sum of the $^{14}CO_2$ and $DO^{14}C$ activities. The fraction of elements released to the dissolved phase decreased from 85% for Cd to 15% for Ag (Fig. 2). The radioactivity in the animals was 20% of the total C, 7% of the total Cd, 3% of the total Po, and negligible amounts for the other metals. These results were compared with the partitioning of radioactivity from duplicate samples of cells incubated with only microorganisms (Fig. 3); the difference between the 2 treatments in the fractions of the elements released to the dissolved phase rarely exceeded 15% of the original label. Copepod grazing enhanced the partial conversion of Ag from the phytoplankton cells to other forms (particulate and dissolved) by 27%, Po by 25%, Pb by 20%, Cd by 13%, and Co by 10% over those cells incubated with only microorganisms. The radioactivities of all the isotopes retained by the cell debris were always lower in the samples treated with microorganisms + copepods than the samples treated with only microorganisms, and most of these differences were attributable to element partitioning into copepods and fecal pellets (Figs. 2 & 3).

Spinning wheel experiment

The fractions of ^{110m}Ag, ^{109}Cd, ^{57}Co, ^{210}Pb and $PO^{14}C$ retained in the decomposing copepod carcasses and of ^{110m}Ag, ^{57}Co, ^{210}Pb and $PO^{14}C$ in the fecal pellets incu-

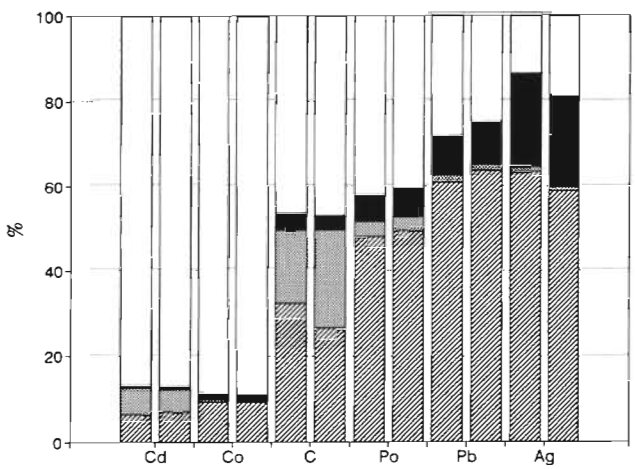

Fig. 2. *Thalassiosira pseudonana, Temora longicornis, Acartia tonsa*. Partitioning in replicate experiments of ^{14}C, ^{110m}Ag, ^{109}Cd, ^{57}Co, ^{210}Pb, and ^{210}Po activity among diatom cells and debris (▨), animal (▩), fecal pellet (■), and dissolved (□) fractions after 40 h incubations of the diatom with copepods

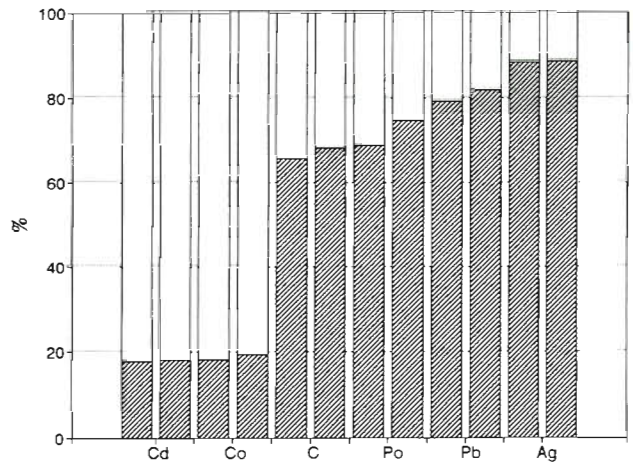

Fig. 3. *Thalassiosira pseudonana*. Partitioning in replicate experiments of ^{14}C, ^{110m}Ag, ^{109}Cd, ^{57}Co, ^{210}Pb and ^{210}Po activity between diatom cells and debris (▨) and dissolved (□) fractions after 40 h incubations of the diatom with microorganisms

bated in the undisturbed vessels at 18 °C or 2 °C or in the vessels placed on a spinning wheel are shown as a function of time (Figs. 4 to 6). Regression analysis of log-transformed data for these elements retained in the debris (log % elements retained vs log time) yielded significant ($p < 0.05$) regression lines (except for Ag in carcasses at 2 °C) according to:

$$y = 100\,(t + 1)^{-b} \quad (1)$$

where y is the percentage of elements retained in the particles, t is incubation time (d), and b is the release rate coefficient (log-log slope). Table 1 presents calculated retention half-times ($t_{r1/2}$) using Eq. (1), release rate coefficients (b) and their 95% confidence limits, and r^2 values of the regression lines.

Carbon loss from copepod debris decreased exponentially over time and carcasses generally lost their carbon faster than did fecal pellets (Fig. 4, Table 1). The most notable difference between the 2 particle types at 18 °C was that the fecal pellets held on the spinning wheel lost their carbon at rates 2 times those of non-sinking fecal pellets (Table 1). Carbon loss from carcasses showed no appreciable difference between these 2 treatments (Table 1).

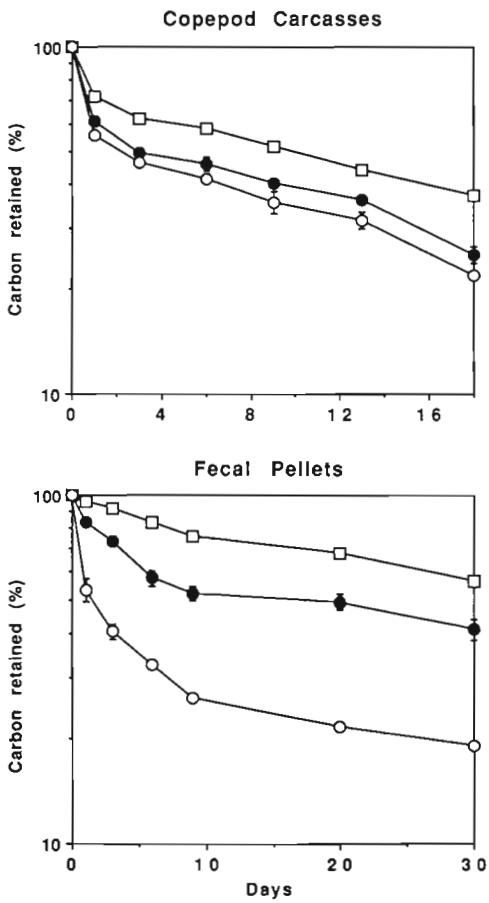

Fig. 4. *Temora longicornis, Acartia tonsa*. Retention in replicate experiments of ^{14}C in copepod carcasses and fecal pellets incubated on a spinning wheel at 18 °C (○), or in a stationary incubator at 2 °C (□) and 18 °C (●). Error bars denote 1 SE; for most samples they were smaller than the symbols for the means

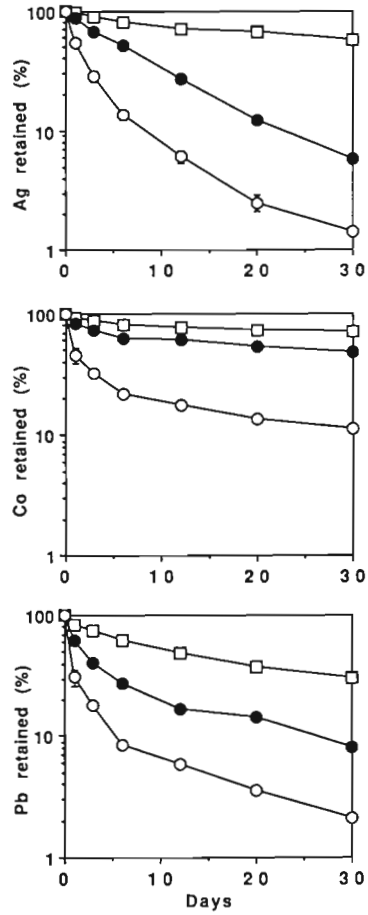

Fig. 5. *Temora longicornis, Acartia tonsa*. Retention in replicate experiments of ^{110m}Ag, ^{57}Co and ^{210}Pb in copepod fecal pellets incubated on a spinning wheel at 18 °C (○), or in a stationary incubator at 2 °C (□) and 18 °C (●). Error bars denote 1 SE; for most samples they were smaller than the symbols for the means

Release of all elements from both types of particles was generally faster at 18 °C than at 2 °C (Figs. 5 & 6, Table 1). The release of ^{110m}Ag, ^{57}Co and ^{210}Pb was much faster from sinking pellets than from non-sinking pellets (Fig. 5). For carcasses, ^{110m}Ag was the only metal whose retention was affected by sinking, and this effect was small (Fig. 6). ^{109}Cd activity in the fecal pellets was too low to monitor. At the end of the incubations, the radioactivity associated with the container walls (determined as in Fisher et al. 1984) was <2% of total ^{14}C, ^{110m}Ag, ^{109}Cd, ^{57}Co and ^{210}Pb for all treatments except for Ag from carcasses (10% at both temperatures), and Co and C from fecal pellets (5% at 18 °C).

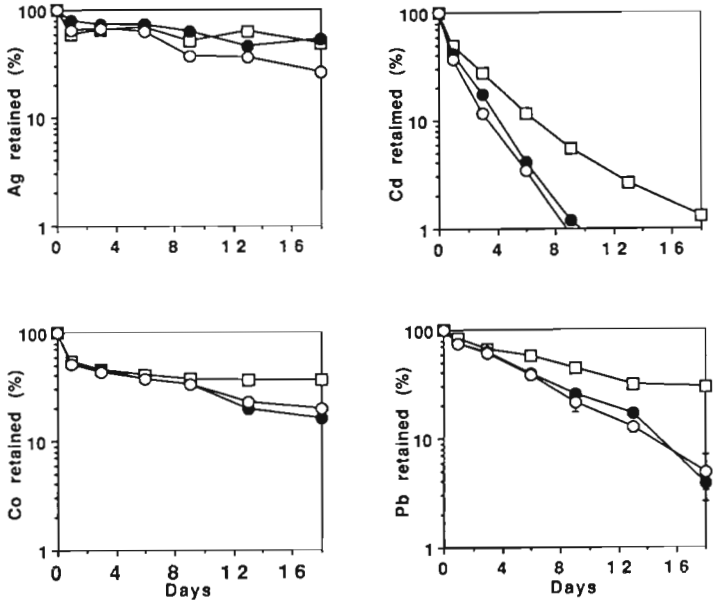

Fig. 6. *Temora longicornis, Acartia tonsa*. Retention in replicate experiments of ^{110m}Ag, ^{109}Cd, ^{57}Co and ^{210}Pb in copepod carcasses incubated on a spinning wheel at 18°C (○), or in a stationary incubator at 2°C (□) and 18°C (●). Error bars denote 1 SE; for most samples they were smaller than the symbols for the means

Table 1. Calculated retention half-times ($t_{r1/2}$) ± standard error (SE), release rate coefficients (b), 95% confidence limits of b, and r^2 values of regression lines [$y = 100(t+1)^{-b}$] describing elemental release from decomposing planktonic debris. 2: 2°C; 18: 18°C; ST: stationary particles; SK: sinking particles. Values in parentheses are b values normalized to values for stationary particles at 18°C

Element		$t_{r1/2}$ ± SE	b	95% CL of b		r^2
Carcasses						
C	18-ST	4.0 ± 0.3	0.429 (1.00)	0.371	0.488	0.925
	18-SK	3.2 ± 0.2	0.482 (1.12)	0.415	0.549	0.920
	2-ST	8.1 ± 0.6	0.314 (0.73)	0.280	0.348	0.955
Cd	18-ST	0.4 ± 0.0	1.930 (1.00)	1.648	2.211	0.943
	18-SK	0.4 ± 0.0	2.076 (1.08)	1.814	2.339	0.955
	2-ST	0.7 ± 0.0	1.309 (0.68)	1.130	1.488	0.948
Pb	18-ST	1.5 ± 0.1	0.746 (1.00)	0.464	1.027	0.741
	18-SK	1.5 ± 0.2	0.765 (1.03)	0.533	0.997	0.814
	2-ST	5.2 ± 0.2	0.381 (0.51)	0.319	0.442	0.930
Co	18-ST	2.3 ± 0.0	0.579 (1.00)	0.497	0.661	0.926
	18-SK	2.6 ± 0.1	0.547 (0.94)	0.475	0.618	0.929
	2-ST	4.3 ± 0.2	0.414 (0.72)	0.322	0.507	0.737
Ag	18-ST	19.5 ± 8.0	0.230 (1.00)	0.182	0.277	0.861
	18-SK	4.8 ± 0.2	0.395 (1.72)	0.309	0.480	0.861
	2-ST	16.0 ± 1.3	0.245 (1.07)	0.156	0.333	0.430
Fecal pellets						
C	18-ST	15.0 ± 3.5	0.250 (1.00)	0.232	0.268	0.983
	18-SK	2.8 ± 0.0	0.520 (2.08)	0.454	0.585	0.933
	2-ST	216.5 ± 74	0.129 (0.52)	0.096	0.162	0.867
Pb	18-ST	1.7 ± 0.0	0.690 (1.00)	0.648	0.731	0.989
	18-SK	0.8 ± 0.0	1.147 (1.66)	1.058	1.235	0.978
	2-ST	8.7 ± 0.0	0.305 (0.44)	0.260	0.350	0.945
Co	18-ST	25.1 ± 0.6	0.212 (1.00)	0.198	0.227	0.983
	18-SK	1.8 ± 0.1	0.681 (3.21)	0.597	0.766	0.936
	2-ST	989.0 ± 163	0.100 (0.47)	0.097	0.104	0.995
Ag	18-ST	2.0 ± 0.0	0.635 (1.00)	0.438	0.833	0.826
	18-SK	0.8 ± 0.0	1.157 (1.82)	1.046	1.269	0.975
	2-ST	169.9 ± 1.3	0.135 (0.21)	0.108	0.162	0.910

DISCUSSION

Carbon release from diatoms and their debris

Zooplankton grazing resulted in the conversion of algal carbon into animal carbon, CO_2, and fecal pellets, with about 35% more carbon removal from the diatoms than generated by microorganisms alone. This is consistent with the idea that zooplankton feeding activities accelerate organic particle degradation by grazing (Conover & Mayzaud 1984, Lampitt 1992, Dam et al. 1993) and leakage of DOM during ingestion and digestion (Jumars et al. 1989, Banse 1990). Copping & Lorenzen (1980) fed *Calanus pacificus* on ^{14}C-labeled diatoms (*Thalassiosira fluviatilis*) for 48 h at 15°C and reported comparable values for ^{14}C activity partitioned in the copepod body (22%) and fecal pellets (3%) but greater values in the cell debris (42%) and DOC (20%), and less in the CO_2 (12%) fractions than in this study. This discrepancy is probably due to the fact that cultures in our study were enriched with microorganisms as well as copepods, and microbial activity enhanced the decomposition of cellular C and conversion of DOC into CO_2. Note that microorganisms remineralized large amounts of cellular C into CO_2, and DOC remained as a small fraction of total carbon (Fig. 1).

The relative role of zooplankton grazing in the removal of phytoplankton biomass largely depends, of course, on the relative biomass and composition of both the herbivorous zooplankton and the phytoplankton. Studies have estimated that zooplankton grazing can account for removal of 1 to 10% (Colebrook 1979, Dam et al. 1993) but as high as 30 to 100% (Conover & Mayzaud 1984, Welschmeyer & Lorenzen 1985) of the daily primary production in various coastal and oceanic surface waters. Although the results from this study may not be directly comparable to others due to differences in the concentration and composition of both food and zooplankton used in these experiments, the results reconfirm the importance of zooplankton grazing on the removal of phytoplankton cells. The extent of zooplankton grazing and its importance to particle degradation in the mesopelagic or bathypelagic zone of the ocean is largely unknown (Banse 1990, Lampitt 1992) due to lack of information on the biomass and behavior of zooplankton (including migratory species) in deeper waters. Moreover, the results acquired from surface waters may not be applicable to deeper water due to differences in the composition of the particles. Banse (1990) estimated from the zooplankton biomass at 100 to 200 m in the Panama Basin and their daily oxygen demand that zooplankton respiration requires 50 to 100% of the POC flux. In bathypelagic waters (5500 m) of the North Atlantic, zooplankton grazing was responsible for 9% of POC remineralization (Lampitt 1992).

Our results suggest that microbial activity is comparable to zooplankton activity in the removal of cellular carbon from 'fresh' phytoplankton cells and debris, at least under the conditions of this experiment. However, some of the carbon loss in cultures with microorganisms was due to physical leaching/chemical dissolution or enzymatic hydrolysis of cell debris, as shown elsewhere (Knauer et al. 1984, Lee & Fisher 1992b, Smith et al. 1992). It is difficult to assess the relative roles of microbial decomposition vs physical leaching/chemical dissolution in the carbon loss from 'fresh' phytoplankton cells because the methods to separate microbial decomposition from physical/chemical processes typically introduce experimental artifacts. Lee & Fisher (1992a) reported that when 'fresh' diatom cells were incubated with various poisons about 40% of total cellular carbon was lost within 2 h. This leaching of cellular carbon was due to damage to phytoplankton cell membranes by the poisons.

Leaching/dissolution of carbon from 'detrital' particles (e.g. fecal pellets) proceeds gradually and the use of poisons does not introduce serious experimental artifacts (Lee & Fisher 1992b). Lee & Fisher (1992b) reported that physical/chemical leaching of DOC was comparable to microbial activity in accounting for carbon loss from copepod fecal pellets incubated at 18°C for 30 d; at 2°C, most of the carbon loss was due to leaching/dissolution and the released DOC was not actively remineralized by microorganisms. Smith et al. (1992) suggested that microorganisms do not directly decompose organic particles and that DOM loss is primarily attributable to enzymatic hydrolysis of POC. Hoppe et al. (1993) hypothesized from extracellular enzymatic activity measurements at the North Atlantic JGOFS stations that enzymatic hydrolysis of particles and bacterial secondary production are well coupled, leading to depletion of easily degradable substrates in deeper waters. Physical/chemical leaching and enzymatic hydrolysis are probably more important than direct microbial decomposition of particles in deep colder waters where particles are more refractory after the labile fractions are utilized in the upper warmer waters.

Application of the results acquired from this study, which used a particular combination of biomass and composition of zooplankton and phytoplankton, may be limited. In the sea, a broad spectrum of zooplankton interact dynamically with a diverse phytoplankton community. Thus, inclusion of other components of the food chain such as protozoans, as well as using different biomass and composition of both zooplankton and phytoplankton, and employing different incubation periods might have produced different experimental results (see also Hutchins & Bruland 1994, this issue).

Metal release from diatom debris

The retention of metals in the diatoms and their debris incubated with only microorganisms was comparable to that in previous studies with *Thalassiosira pseudonana* (Lee & Fisher 1992a, 1993a, Fisher & Wente 1993). The emerging trend from these studies is that microorganisms play a major role in the release of those elements (e.g. Cd, Se and Zn) which penetrate into the cytoplasm of phytoplankton cells (Reinfelder & Fisher 1991). Release rates of these elements are typically most pronounced in the early stage of particle decomposition and decrease exponentially over time, following the rates of carbon and protein release (Lee & Fisher 1993a). As discussed earlier, physical/chemical leaching also seems to be an important release mechanism for these elements during early stages of decomposition. Particle-reactive elements (including Ag, Am, Ce, Pb and Th) which associate primarily with structural components of phytoplankton cells (Fisher et al. 1983c, Reinfelder & Fisher 1991) are retained longer than are carbon and protein as particles decompose, and microbial activities have a negligible influence on their release (Lee & Fisher 1992a, 1993a).

The conversion of elements from phytoplankton cells and debris to other fractions (from 10% for Co to 27% for Ag) by zooplankton is due to ingestion and subsequent assimilation by the animals of some elements (e.g. Cd) associated with the food and defecation of unassimilated elements in fecal pellets (Ag, Pb and Po). As a result of grazing, the release of elements from particles into the dissolved-phase fraction increased by 5 to 15%. Release of elements from decomposing fecal pellets probably also contributed to some of the elements in the dissolved fraction (Lee & Fisher 1992b). Hutchins & Bruland (1994) demonstrated in experiments with different zooplankton and phytoplankton assemblages that zooplankton can release and assimilate Fe, Mn and Zn from diatom and protozoan food and concluded that zooplankton grazing is the dominant process recycling these metals in the euphotic zone.

Reinfelder & Fisher (1991) demonstrated that assimilation efficiency of ingested food-associated elements by copepods directly correlates with the fraction of elements associated with the cytoplasm of the diatom *Thalassiosira pseudonana*. In marine copepods, assimilation efficiencies of 97% for Se, 84% for C, 48% for Zn, 30% for Cd, 21% for Hg, 17% for Ag and only 1 to 4% for Pu and Am have been measured (Fisher et al. 1991a, Reinfelder & Fisher 1991). In the diatom *T. pseudonana*, <1% of ^{210}Pb and 30% of ^{210}Po penetrate into the cytoplasm (Fisher et al. 1983c). Consistent with earlier studies which demonstrated ^{210}Po enrichment in marine invertebrates, we found that about 2 times more Po (5 times more Cd) than Pb was retained by the copepods. Those elements bound largely to structural components of the cells (e.g. Ag and Pb) were mostly released in fecal pellets.

Lower retention of Co by the copepods in this study is consistent with the findings of Nolan et al. (1992) that phytoplankton preferentially accumulate Co-cobalamine over inorganic Co and that Co-cobalamine is assimilated in copepods with much greater efficiency than is inorganic Co. Low biological cycling of Co together with microbial oxidation of Co into more insoluble oxides (Tebo et al. 1984, Lee & Fisher 1993b) help to explain its scavenged-type vertical profile in the water column (Knauer et al. 1982).

Oceanographic observations show that ^{210}Po/^{210}Pb ratios increase from surface waters (<1) to midwater depths (>1) and remain close to 1 in deeper waters (Bacon et al. 1976, Cochran et al. 1983). ^{210}Po has a residence time of about 0.6 yr in the surface ocean and is believed to be affected by biological removal from surface waters and regeneration below the euphotic zone (Bacon et al. 1976, Nozaki & Tsunogai 1976, Beasley et al. 1978, Cochran et al. 1983). Cd displays nutrient-type vertical profiles, analogous to phosphate, presumably due to its incorporation into organisms in surface waters and regeneration at depth (Boyle et al. 1976). Sediment trap studies have shown that Cd is more enriched in biogenic particles than in non-biogenic particles and its flux decreases rapidly with depth, similar to phosphate and organic matter (Noriki & Tsunogai 1992). The Ag results from this study are consistent with other laboratory studies (Lee & Fisher 1992a, Fisher & Wente 1993, Reinfelder et al. 1993) which show long retention of Ag in decomposing phytoplankton debris and marine snow.

The release of elements such as Cd, Se, Zn and Po which enter the cytoplasm of phytoplankton cells is largely governed by microbial activity. It would appear from this study that zooplankton grazing activity slightly enhances the recycling of these elements in surface waters. Consequently, these elements have long residence times in surface waters and their flux decreases with depth. Microbial activity has a relatively minor influence on the release of those elements associated with structural components of cells, but zooplankton may play a major role in removing these elements by grazing on the cells and packaging these unassimilated elements into rapidly sinking fecal pellets. These elements typically have long retention times in fecal pellets and are not appreciably affected by microbial decomposition (Lee & Fisher 1992b). Consequently, they are removed from surface waters and are transported to depth, leading to relatively short residence times in the surface ocean.

Sinking vs stationary particles

The greater elemental loss and carbon degradation from sinking fecal pellets on a spinning wheel than from non-sinking pellets was probably due to both the reduction of the boundary layer of released material surrounding decomposing fecal pellets and the increased fragmentation of fecal pellets during constant sinking. With a sharp concentration gradient of released material around free-falling fecal pellets, the diffusion of trace elements and organic carbon contained in fecal pellet matrices as unassimilated digestive byproducts or released due to microbial decomposition and solubilization should be unimpeded.

Microscale chemical patches are found inside zooplankton fecal pellets and marine snow; these particles contain metabolically active species of aerobic and anaerobic bacteria which accelerate the solubilization and release of particulate organic matter (Gowing & Silver 1983, Alldredge & Cohen 1987, Bianchi et al. 1992). Constant sinking of particles could increase the removal of microbial metabolic waste products by diffusion from the interior of fecal pellets and accelerate diffusion of O_2 into the interior of fecal pellets, leading to faster decomposition and solubilization of the pellets. Additionally, with greater particle degradation and material loss, fecal pellets may lose their peritrophic membrane (and consequently the integrity of compacted cell fragments) and as a result the contents of the fecal pellets would be released into the surrounding water. Jumars et al. (1989) suggested from a model calculation that the diffusion of solutes contained in fecal pellets was insensitive to fluid dynamic conditions (e.g. free-falling vs stationary), and that most solutes diffuse out of fecal pellets within several minutes. A possible explanation for the discrepancy between the results of this study and the conclusion of Jumars et al. is that the rate of degradation of the peritrophic membrane encapsulating fecal pellets held on a spinning wheel increased due to more rapid decomposition. It is also possible that particles incubated on the spinning wheel collided with container walls and thus accelerated particle fragmentation via this experimental artifact.

Shanks & Edmondson (1989) observed in the rotation of cylindrical tanks containing unfiltered seawater that the particles in the seawater formed aggregates resembling marine snow. Similarly, microscopic observations of both decomposing copepod fecal pellets and carcasses revealed that both particle types held on the spinning wheels in our study coagulated to form large aggregates while individual particles remained separated from each other in the non-sinking debris.

No appreciable difference in the rates of elemental loss and degradation was observed between copepod carcasses in free-falling and stationary containers. Degradation of copepod carcasses generally proceeds with a rapid leaching of soluble internal organic pools in the early stage of decomposition (Harding 1973, Lee & Fisher 1992b), during which most of the carbon and trace elements are lost. Fluid dynamics apparently have little influence on the decomposition of the remaining refractory material, including the chitinous exoskeleton.

The results from this study suggest that the rates of elemental loss and particle decomposition from free-falling particles are somewhat faster than those from stationary particles settled on the bottom of experimental vessels. To evaluate this issue further, problems associated with spinning wheels such as collision of particles with incubator walls and the accumulation of released material in small volumes of solution need to be explored.

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