Grazer-mediated regeneration and assimilation of Fe, Zn and Mn from planktonic prey

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ABSTRACT: Experiments were performed to investigate grazer remineralization and assimilation of Fe, Zn and Mn from autotrophic and heterotrophic plankton prey. Metal isotope activity incorporated into planktonic prey was added to bottles containing crustacean zooplankton grazers, and distribution of the added metals into dissolved, fecal pellet and grazer fractions was monitored over time. At the end of a 9 to 10 h grazing period, concentrations of dissolved metal isotopes were approximately 3 to 7 times higher in bottles with grazers than in control bottles without grazers. An experiment in which flagellate grazers were fed Fe-labeled cyanobacteria suggested that protozoans may also remineralize trace metals ingested with prey. Metal assimilation efficiencies from diatom and flagellate prey were determined in crustacean grazers; efficiencies generally decreased in the order Zn > Fe > Mn. These experiments indicate that biologically required trace metals behave much like major nutrients during grazing, and suggest that biologically mediated regeneration and recycling could be an important part of the marine biogeochemical cycles of Fe, Zn and Mn.

KEY WORDS: Regeneration · Remineralization · Trace metals · Iron · Zinc · Manganese · Zooplankton · Grazing

INTRODUCTION

The cycling of nutrients in oceanic euphotic zones is driven largely by phytoplankton assimilation and regeneration by grazers. Some portion of the organic material produced is exported from the surface layer and remineralized in the underlying water column. The combination of these biologically mediated processes results in characteristic nutrient-type vertical profiles for nitrate, phosphate and silicic acid.

The marine biogeochemistry of some trace elements such as zinc and cadmium is also dominated by biological cycling within the marine system (Bruland 1983, Sunda & Huntsman 1992), causing these elements to exhibit nutrient-type profiles. By contrast, cycling of more particle-reactive metals such as iron and manganese has usually been assumed to be dominated by abiotic processes such as external input and removal by adsorption onto sinking particles.

Recent interest in trace metals, particularly iron, has been been focused on their potential to act as limiting nutrients in some open ocean regions (Martin et al. 1991). Because elements such as Fe, Mn and Zn are required for various biochemical functions, it has been argued that inadequate supplies of these metals relative to the supply of major nutrients could constrain biological productivity (Brand et al. 1983, Martin et al. 1991).

Elemental ratios of plankton samples suggest that Fe is required in the largest amounts of any 'trace' metal, followed closely by Zn, with Mn and others present in lesser amounts (Bruland et al. 1991). This perspective, in which trace metals are recognized as required nutrients, has led to the question of whether they may be actively regenerated and recycled by biological activity in the euphotic zone, as is the case for major nutrients.

Hutchins et al. (1993) recently demonstrated that iron can be rapidly recycled from labeled living phyto-

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plankton cells added to natural communities. Despite the fact that iron is chemically a hydrolyzed, highly particle-reactive element, it appears that there is a potentially important biologically mediated, regenerated component to the marine iron cycle. The combination of active biological uptake and regeneration together with passive scavenging may explain the fact that this trace metal exhibits a vertical profile which has characteristics of both nutrient-type and scavenged elements (Bruland et al. 1991).

Although Hutchins et al. (1993) demonstrated the ability of plankton communities to recycle iron, their experiments did not differentiate between various possible transfer mechanisms. Like Frey & Small (1979), they hypothesized that remineralization by grazers could be an important route for regeneration of iron, just as it is for major nutrients. This hypothesis was tested in the series of laboratory experiments presented here, in which grazer-mediated remineralization and assimilation of cellular Fe, Mn and Zn were measured.

MATERIALS AND METHODS

This set of experiments investigated the fate of metal isotopes (Fe, Zn and Mn) previously incorporated into prey cells (cultured diatoms, protozoans or cyanobacteria) during grazing by crustacean or protozoan zooplankters. The trophic interactions for which remineralization and/or assimilation efficiencies were determined are illustrated in Fig. 1. Isotope activity was monitored over time in the dissolved fraction, as well as in fecal pellets and in the animal grazers themselves. In addition, at the end of most of the grazing experiments, metal isotope assimilation efficiencies were determined after a depuration period.

Expts 1 to 4 were designed to investigate the fate of metals incorporated into diatom or protozoan prey cells during grazing by several different species of crustacean zooplankton. Expt 5 examined remineralization of Fe from cyanobacterial prey by protozoan grazers. Initial conditions for all the grazing experiments are presented in Table 1, including grazer species and density, prey species and density, and activities of Fe, Zn or Mn isotopes added to each replicate bottle as prey. Controls for all the experiments consisted of identical concentrations of labeled prey cells added to the same seawater used in the grazing bottles, but with no grazers present. This allowed differentiation of grazing-mediated isotope release from spontaneous release due to desorption, cell lysis or other processes.

All grazing experiments were carried out at 12 °C with moderate shaking to keep the cells suspended. Seawater used in all grazing and control bottles was 0.2 μm
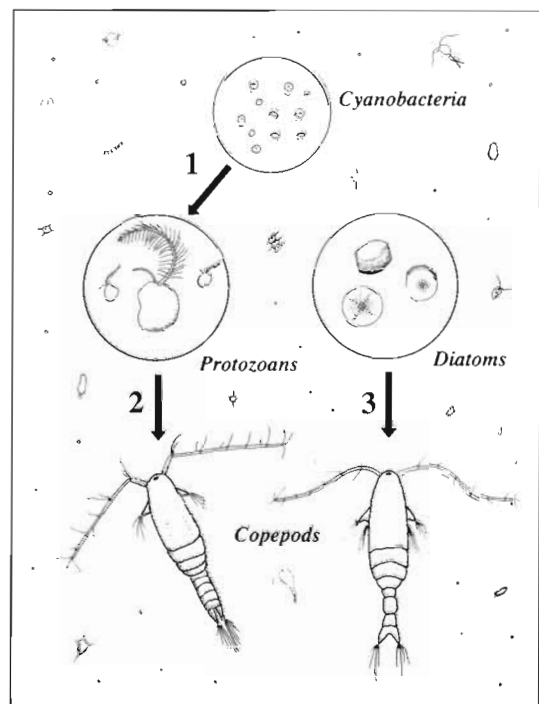

Fig. 1. Experiments measured regeneration and/or assimilation efficiencies of biologically required trace metals (Fe, Zn, Mn) for the trophic interactions illustrated here: (1) picoplankton (*Synechococcus* CCMP 1334) as prey for protozoan grazers (*Paraphysomonas* strain CL2), Expt 5; (2) Protozoa as prey for crustacean grazers (copepods and cladocerans), Expts 3 & 4; and (3) diatoms (*Thalassiosira weissflogii*) as prey for crustacean grazers: brine shrimp in Expt 1, copepods and cladocerans in Expts 2, 3 & 4

filtered, UV-oxidized seawater from which trace metals had been removed by passage through a Chelex-100 ion exchange column (UVSW) (Donat & Bruland 1988). Expts 1 to 4 were carried out in the dark to minimize isotope reassimilation by prey during the 9 to 10 h grazing period (see Goldman et al. 1985). Because Expt 5 was monitored for an extended period (8 d), bottles were incubated in the light to allow the cyanobacteria prey to remain viable for the duration of the experiment. Experimental bottles were acid-washed polycarbonate flasks containing 250 to 1000 ml UVSW and varying densities of grazers and prey (Table 1). All reported values are the mean of replicate bottles.

Periodically during the feeding experiments, samples were removed from the bottles for determination of dissolved, grazer and fecal pellet activities. Concentrations of dissolved isotopes were determined by counting the gamma activity of 40 ml of 0.2 μm filtrate (Expts 3 & 4) or liquid scintillation counting of 5 ml of 3 μm filtrate (Expt 1) or 0.2 μm filtrate (Expt 5). Although metal isotope activity passing through filters can include chemical species not truly dissolved (i.e. colloids and some

Table 1. Initial conditions for Expts 1 to 5, including grazer and prey densities and activities of metal isotopes added to each replicate bottle as prey cells

Expt	Grazer (density)	Prey (growth phase, cell density)	Isotopes (kBq added as prey)		
1	Brine shrimp *Artemia* sp. (0.1 ml^{-1})	Diatom *Thalassiosira weissflogii* (stationary phase, $1.5 \times 10^5 \text{ ml}^{-1}$)	^{55}Fe (212)		
2	Copepod (unidentified) (0.5 ml^{-1})	*T. weissflogii* (stationary phase, $2.0 \times 10^5 \text{ ml}^{-1}$)	^{55}Fe (104)		
3	Copepod *Acartia tonsa* (0.2 ml^{-1})	*T. weissflogii* (log phase, $2.1 \times 10^5 \text{ ml}^{-1}$) Flagellate *Paraphysomonas* sp., strain CL2 (log phase, $1.9 \times 10^5 \text{ ml}^{-1}$)	^{59}Fe (2.0) (1.0)	^{65}Zn (2.0) (1.3)	^{54}Mn (2.8) (1.1)
4	Copepod *A. tonsa* and cladoceran *Evadne* sp. (0.4 ml^{-1})	*T. weissflogii* (stationary phase, $2.8 \times 10^5 \text{ ml}^{-1}$) Flagellate *Paraphysomonas* sp., strain CL2 (stationary phase, $2.1 \times 10^5 \text{ ml}^{-1}$)	^{59}Fe (5.5) (1.2)	^{65}Zn (7.2) (1.6)	^{54}Mn (4.3) (1.4)
5	Flagellate *Paraphysomonas* sp., strain CL2 ($2.0 \times 10^4 \text{ ml}^{-1}$)	Cyanobacterium *Synechococcus* sp. CCMP1334 (stationary phase, $1.4 \times 10^6 \text{ ml}^{-1}$)	^{55}Fe (72)		

bacterial cells), in subsequent discussion 3.0 µm filtrate activity (Expt 1) or 0.2 µm filtrate activity (Expts 3, 4 & 5) will be referred to as 'dissolved'.

Grazers in Expt 1 were brine shrimp (*Artemia* sp.) obtained from commercial sources. Prey in this experiment was the centric diatom *Thalassiosira weissflogii* labeled with ^{55}Fe (980 kBq). Prey cells were concentrated by centrifugation (10 min, $730 \times g$) and washed by being resuspended in UVSW to remove residual dissolved isotope activity. This process was carried out 3 times before the labeled cells were resuspended in UVSW; this suspension was then subdivided equally into experimental and control bottles (Table 1).

At hourly intervals, 3 µm filter activity (prey activity) and 3 µm filtrate activity (dissolved activity) were determined using liquid scintillation counting (LSC) techniques as described in Hutchins et al. (1993). Assimilation efficiencies and fecal pellet radioactivities were determined as outlined below, except that activity was determined using LSC. Expt 2 used copepods collected from Monterey Bay, USA (unidentified species) grazing on diatoms labeled with ^{55}Fe (550 kBq); only assimilation efficiencies were measured in this particular experiment.

Natural grazer assemblages were obtained for Expts 3 & 4 from Monterey Bay nearshore surface water with a 333 µm mesh net. Grazers were held for about 6 h in UVSW before being used in experiments. Expt 3 used a monospecific collection of the copepod *Acartia tonsa*; the grazers in Expt 4 were a mixed assemblage of *A. tonsa* and a cladoceran, *Evadne* sp., in a ratio of approximately 2:1.

Prey in Expts 3 & 4 consisted of the diatom *Thalassiosira weissflogii* (CCMP strain 1336), or a flagellated

protozoan (*Paraphysomonas* sp., strain CL2, obtained courtesy of Joel Goldman at Woods Hole Oceanographic Institute). We used both phytoplankton and protozoan cells as prey, as both these groups can be significant food sources for crustacean grazers in the ocean (Stoecker & Capuzzo 1990). Experiments were carried out using labeled diatom and flagellate cultures in both exponential and stationary growth phases, since previous work has demonstrated that there can be significant differences in grazer assimilation of trace metals based on growth stage of the prey (Reinfelder & Fisher 1991).

The diatom species used as prey in these experiments has a cell diameter of 7 to 10 µm, and the flagellate species (used as prey in Expts 3 & 4 and as grazer in Expt 5) has a cell diameter of 4 to 5 µm. Protozoan numbers were monitored by visual cell counts, while changes in diatom cell numbers were followed using *in vivo* fluorescence or cell counts.

Diatom cultures were radiolabeled by addition of gamma-emitting isotopes (^{59}Fe, ^{65}Zn and ^{54}Mn) to UVSW enriched with Aquil nutrients and trace metals (Price et al. 1989). Cultures were grown under continuous cool white fluorescent lights at a photon flux density of 100 µmol quanta m^{-2} min^{-1}. Log-phase cultures were harvested after 3 d in the labeling medium, and stationary phase cultures were used after 10 d. Diatom cells were concentrated and washed as outlined above before being added to the experimental bottles; prey activities and cell concentrations added to each bottle are given in Table 1.

Flagellate cultures were radiolabeled for Expts 3 & 4 by feeding stock cultures with cyanobacteria (*Synechococcus* sp., CCMP strain 1334) which had been

grown in the same labeling medium used for the diatom cultures. Log-phase cells were used after 3 d of feeding on the labeled cyanobacteria, and stationary phase cultures after 13 d. Protozoan cultures were added to feeding suspensions without centrifugation or concentration to avoid damage to the cells. Epifluorescence microscopic examination revealed that virtually all the labeled cyanobacteria had been grazed from the stationary-phase flagellate cultures before these cultures were used for feeding experiments. The log-phase flagellate cultures, however, still contained labeled cyanobacteria cells at the time they were used. It was assumed that the crustacean grazers used were grazing mainly on the added protozoans, not the cyanobacteria, as most metazoan grazers generally do not efficiently utilize picoplankton cells (1 µm diameter) as a food source (Johnson et al. 1982, Wylie & Currie 1991).

In Expts 3 & 4, 10^{-4} M EDTA was added to both grazing bottles and controls as a 'dissolved trap' to discourage any potential scavenging of remineralized isotopes onto fecal pellets, prey cells or other surfaces (see 'Discussion'). Expts 1, 2 & 5 used UVSW without any added metal chelator.

Activity of grazers was determined by pipetting out individual animals or by gravity filtration onto 333 µm Nitex. Animals were rinsed with UVSW before counting to remove carry-over activity. The concentration of grazers was kept constant throughout the experiment by removing animals in proportion to total volume removed. Fecal pellet activity was obtained by filtering 50 ml samples from which grazers had been removed onto 64 µm Nitex.

At the end of the feeding period, grazers were removed and allowed to depurate for 18 h in UVSW with concentrations of unlabeled prey similar to those in the experimental bottles (to facilitate gut evacuation). Assimilation efficiencies were determined as in Reinfelder & Fisher (1991). Briefly, assimilation efficiency was defined as activity per rinsed animal at the end of the depuration period divided by activity ingested per animal during the feeding period (determined by multiplying numbers of cells removed by grazing by activity per cell). Replicates of 10 to 50 animals were used in assimilation efficiency analyses.

Activity of gamma-emitting isotopes was determined by counting on a Ge(Li) detector. A 2 h counting period was used because activity in some samples was low; propagated counting errors ranged from < 1 % to about 12 %. Standards in the appropriate geometries were used to convert sample peak areas to activities. Due to the long counting times necessary for these samples, only an initial, final and 1 midpoint were obtained for time-course measurements of gamma activity counts (Expts 3 & 4).

Expt 5 was designed to demonstrate whether protozoan grazers (*Paraphysomonas* sp. strain CL2) remineralize iron ingested with prey. Prey in this experiment was the cyanobacterium *Synechococcus* sp. CCMP 1334 labeled with ^{55}Fe (330 kBq). Iron activity in the dissolved phase (0.2 µm filtrate) was monitored over a period of 8 d using LSC. Grazer and pellet activities and assimilation efficiencies were not measured in this experiment due to the difficulty of reproducibly separating protozoan cells from labeled prey cells.

RESULTS

The results of Expt 1, in which *Artemia* sp. grazers were fed diatoms labeled with ^{55}Fe, are presented in Fig. 2. *In vivo* fluorescence measurements indicated that virtually all the diatoms were removed by the brine shrimp over the 10 h course of this experiment (Fig. 2A). The slope of this graph corresponds to an initial clearance rate of 0.8 ml animal^{-1} h^{-1}. Fig. 2B illustrates the increase in dissolved ^{55}Fe activity in the 3 µm filtrate during the grazing period. At the final time-
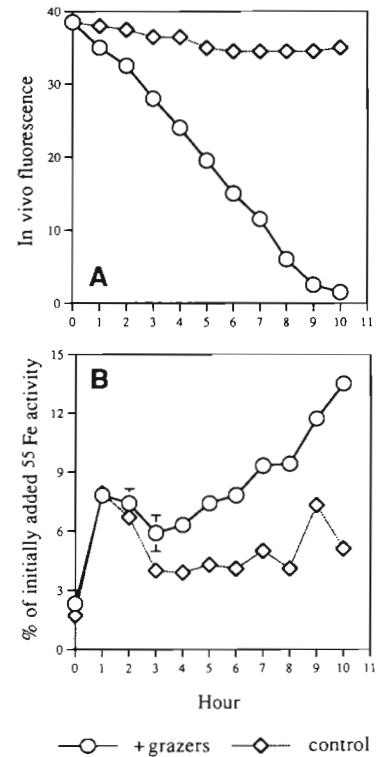

Fig. 2. Expt 1. *Artemia* sp. grazing on *Thalassiosira weissflogii*. (A) *In vivo* fluorescence of diatom prey in controls and in bottles with brine shrimp grazers. (B) Percent of initial ^{55}Fe activity added as diatom prey found in the dissolved fraction (3 µm filtrate) in controls and in bottles with grazers. Error bars represent the range of duplicate bottles
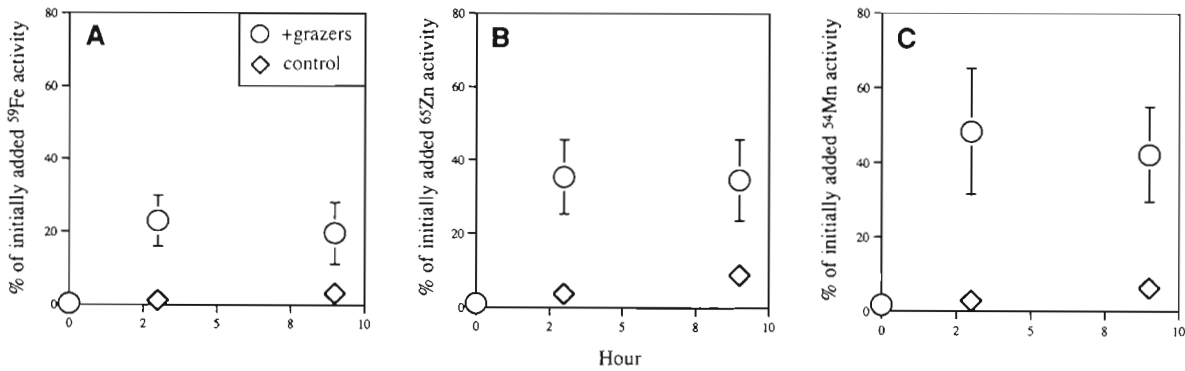
Fig. 3. Expt 3. *Acartia tonsa* grazing on *Thalassiosira weissflogii*. Percent of initial isotope added as diatom prey found in the dissolved fraction (0. 2 μm filtrate) in controls and in bottles with copepod grazers. Error bars represent the range of duplicate bottles. (A) ^{59}Fe; (B) ^{65}Zn; (C) ^{54}Mn

point, dissolved Fe activity levels in the bottles with brine shrimp were almost 3 times greater than in the control bottles (labeled diatoms without grazers). Both the control and the grazer bottles exhibited a rapid rise in dissolved activity during the first hour of the experiment, suggesting a fast initial release of some of the label from the phytoplankton cells similar to that reported by Reinfelder & Fisher (1991) and Lee & Fisher (1993) for other trace metals.

Measurements of ^{55}Fe activity at Hour 10 in Expt 1 indicated that most of the added isotope (69%) was associated with fecal pellets at the end of the experiment. The amounts of ^{55}Fe in the dissolved phase (14%) and associated with the grazers (13%), however, also represented a significant fraction of the originally added activity. Only 4% of the added ^{55}Fe was still associated with the size fraction containing the diatom prey (3 to 64 μm fraction) at the end of the experiment, consistent with the almost complete removal of prey suggested by the *in vivo* fluorescence data (Fig. 2A).

The results of Expts 3 & 4, in which Monterey Bay zooplankton assemblages were fed diatoms labeled with ^{59}Fe, ^{65}Zn and ^{54}Mn, demonstrate rapid remineralization of cell-associated trace metals during grazing (Figs. 3 & 4). Final dissolved metal isotope concentrations in the grazing bottles comprised 7 to 42% of the total activity originally added as prey, and exceeded the amount remineralized in the control bottles (2 to 9%) by about 3 to 7 times. A greater proportion of the total isotope activity was remineralized from log-phase diatom prey (17 to 36% over controls; Fig. 3) than from stationary-phase prey (5 to 15% over controls; Fig. 4). This is consistent with the higher assimilation efficiencies we measured from stationary-phase diatom prey (see below). In contrast to Expt 1, dissolved isotope levels in the controls increased only slowly and almost linearly, without the rapid initial release observed in the previous experiment.

Increases in dissolved activity of all 3 elements were also observed using labeled flagellates as prey, although they were less dramatic and generally exhib-
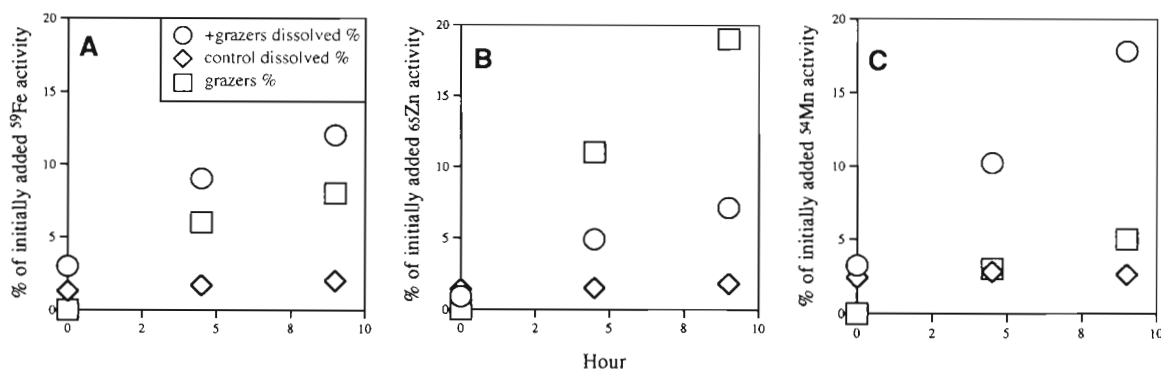

Fig. 4. Expt 4. *Acartia tonsa* and *Evadne* sp. grazing on *Thalassiosira weissflogii*. Percent of initial isotope activity added as diatom prey found in the dissolved fraction (0.2 μm filtrate) in controls and in bottles with copepod and cladoceran grazers. Percent of initially added activity found in the grazers is also shown. Error bars have been omitted for clarity. (A) ^{59}Fe; (B) ^{65}Zn; (C) ^{54}Mn

ited a lag period before dissolved activity in the grazer bottles exceeded that in the controls (data not shown). Because labeled flagellate cultures were not concentrated or washed before being added to the experimental bottles, total activity added as prey cells was somewhat lower than in the bottles with diatom prey (Table 1), and initial dissolved activity was proportionately higher. These factors may account for the smaller increases in dissolved activity observed using protozoans as prey.

Because grazers obtained from the net tows were smaller than the brine shrimp used in the previous experiment, lesser numbers of the labeled diatoms were removed by grazing in the course of these later experiments. Cell counts indicated that about 32% of the diatom cells were grazed out in Expt 3 and 31% in Expt 4, corresponding to clearance rates of 0.18 and 0.09 ml animal^{-1} h^{-1}, respectively. Although an excess of prey has the advantage of allowing more uniform grazing rates over the course of these experiments, this also means that much of the activity originally added with the prey cells was still present in this pool at the end of the experiment.

Final partitioning of the activity of the 3 isotopes in Expts 3 & 4 also differed in other ways from that observed for ^{55}Fe in Expt 1. Fecal pellet activity at the end of the 9 h grazing period was much less important proportionately. For instance, only 2% of the ^{65}Zn, 1% of the ^{54}Mn and 3% of the ^{59}Fe originally added were associated with fecal pellets at the termination of Expt 3. For Expt 4, these numbers were 2%, 1% and 8% respectively. The fact that fecal pellet activities were low may be partly due to complexation of the isotopes by EDTA added to the grazing suspensions in Expts 3 & 4 (see 'Discussion').

In these experiments, incorporation of label into grazers was in most cases a more significant sink for isotope activity than were fecal pellets. Copepod activity before depuration at the end of Expt 3 (log-phase diatom cells) accounted for 10% of the ^{65}Zn, 3% of the ^{54}Mn and 1% of the ^{59}Fe originally added to the feeding suspensions. For grazers fed the stationary-phase diatoms used in Expt 4, these proportions were somewhat higher, comprising 19%, 5% and 7% of the initial activity respectively.

In all cases in Expts 3 & 4, with the sole exception of Zn in Expt 4, dissolved activity was significantly higher than activity in either the fecal pellet or grazer fractions. Dissolved Fe, Zn and Mn accounted for 20%, 35% and 42% of the original activity at the end of Expt 3. In Expt 4,

these same 3 isotopes represented 12%, 7% and 18% of the activity added respectively. These proportions likely represent an upper limit to the amount of each element actually remineralized during this grazing experiment, due to the possibility of solubilization of some fecal pellet-associated metals by added EDTA.

Assimilation efficiencies for all 3 of these experiments are presented in Table 2, along with results of Expt 2, in which unidentified Monterey Bay copepods were fed ^{55}Fe-labeled diatoms. A comparison of assimilation efficiencies of the 3 metals makes it evident that Zn was generally assimilated most efficiently (12 to 56%), Fe next most efficiently (10 to 22%), and Mn least efficiently (2 to 10%).

Results of Expt 5, in which protozoan grazers were fed ^{55}Fe-labeled cyanobacteria, are presented in Fig. 5. The 0.2 µm filtrate activity in the bottles with grazers consistently exceeded that in the control bottles. Although these results suggest remineralization of iron similar to that observed with crustacean grazers, the possibility of isotope release due to lysis of fragile protozoan cells during filtration cannot be excluded (Taylor & Lean 1981, Nagata & Kirchman 1990). An attempt was made to minimize this problem by using vacuum pressures that were as low as practical (10 to 15 kPa); however, at present, the evidence for protozoan remineralization of picoplankton iron remains tentative.

DISCUSSION

These experiments demonstrate that zooplankton can rapidly remineralize Fe, Zn and Mn from plank-

Table 2. Assimilation efficiencies for metal isotopes added as prey in Expts 1 to 4

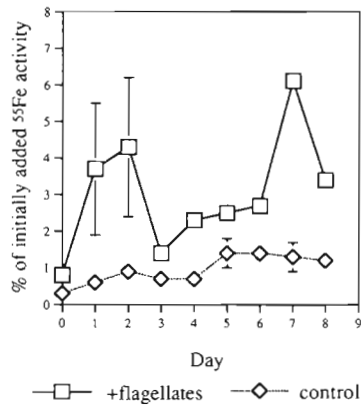| Expt	Grazer	Prey	Isotope	Assimilation efficiency (%)
1	Brine shrimp Artemia sp.	Diatom (stationary phase)	^{55}Fe	19
2	Copepod (unidentified)	Diatom (stationary phase)	^{55}Fe	20
3	Copepod Acartia tonsa	Diatom (log phase)	^{59}Fe	7
			^{65}Zn	37
			^{54}Mn	10
		Flagellate (log phase)	^{59}Fe	18
			^{65}Zn	32
			^{54}Mn	3
4	Copepod A. tonsa and cladoceran Evadne sp.	Diatom (stationary phase)	^{59}Fe	22
			^{65}Zn	56
			^{54}Mn	12
		Flagellate (stationary phase)	^{59}Fe	10
			^{65}Zn	12
			^{54}Mn	2

Fig. 5. Expt 5. *Paraphysomonas* sp. grazing on *Synechococcus* sp. Percent of initial [55]Fe activity added as cyanobacteria prey found in the dissolved fraction (0.2 µm filtrate) in controls and in bottles with protozoan grazers

tonic prey. Oceanographers have long recognized the ability of zooplankton to recycle nutrient elements such as nitrogen or phosphorous from ingested prey. Nutrient remineralization by grazers in surface waters allows re-use of these elements to support continuing cycles of regenerated primary production. An individual nitrogen or phosphorous atom may thus be recycled numerous times before being removed from the surface photic zone with sinking particles. The experiments presented here suggest that grazing could also be important in facilitating regeneration and recycling of biologically required trace metals, in a manner entirely analogous to major nutrient cycling.

Although these experiments indicate that zooplankton grazing does regenerate cellular trace metals, it is difficult to draw quantitative conclusions about *in situ* remineralization rates or efficiencies based on laboratory experiments. Unnaturally high densities of both grazers and prey in experimental bottles can lead to artifactual bias in estimates of zooplankton nutrient release (Lehman 1980). The relatively high cell densities and particulate trace metal concentrations used in these experiments are closer to those often observed in neritic waters than to open ocean values. Differences in biological trace metal cycling may be characteristic of different marine regimes, due to variations in both the biological community and in trace metal supply rates and concentrations. Hutchins et al. (1993) observed that Fe recycling appeared to be more efficient in the low-Fe regime of the equatorial Pacific than in samples from coastal Monterey Bay, an area of high Fe input.

An abundance of prey can lead to less efficient grazer assimilation of ingested nutrients ('gluttonous feeding'), resulting in a greater degree of incorporation of partially digested material into fecal pellets and

possibly higher remineralization rates. Proportions of ingested elements that are found in these fractions can thus be higher than would be the case at the lower prey densities usually encountered in nature (Butler et al. 1970, Gaudy 1974). Alternatively, rapid uptake of remineralized nutrients by remaining prey cells can lead to underestimates of nutrient release by zooplankton (Lehman 1980). Although most of the grazing experiments presented here were performed in the dark to discourage growth and reassimilation by photoautotrophic prey, substantial dark uptake of nutrients such as ammonium by phytoplankton has been reported (Goldman et al. 1987). Whether dark assimilation of remineralized trace metals occurs is unknown.

The fraction of isotopes regenerated to the dissolved phase in grazing bottles ranged from 7 to 42 % of the total activity added; the percentage of total activity remineralized in the grazing bottles and the amount released by cells in control bottles differed by a factor of 3 to 7. In Expt 3, dissolved activity reached final levels as high as 42 % of the total Mn activity added as prey cells (Fig. 3). In this case, the percentages of metals remineralized to the dissolved fraction actually exceeded the fraction of diatom prey consumed in this experiment as estimated by cell counts (32 %). However, cell counts of control bottles in this experiment revealed that the log-phase diatom prey continued to grow after being placed in the darkened experimental bottles, presumably utilizing photosynthate previously stored during growth in the labeling medium. About 0.3 doublings of the diatom prey were measured during the grazing period; thus more cells were consumed in the grazing bottles than was apparent from the cell counts. This may explain the apparent discrepancy between prey consumed and isotope remineralized; if this is the case, assimilation efficiencies for log-phase prey may also be overestimated (see below). The fact that only minor increases in dissolved activity were observed in controls suggests that increases in bottles with grazers were primarily due to grazing, and not to desorption, lysis or bacterial remineralization of phytoplankton trace metals.

Lee & Fisher (1994) present the results of a similar study in which the release of a suite of mostly non-essential trace metals (Ag, Cd, Co, Po and Pb) from phytoplankton debris was examined in the presence and absence of microorganisms and/or copepods. They concluded that microbial degradation processes can account for the bulk of trace metal regeneration in the ocean, with zooplankton grazing accounting for a fractional enhancement (5 to 15 % over a 40 h incubation).

Microbial regeneration may also have contributed to the remineralization observed in these experiments,

since prey cultures and grazer collections were not axenic. In spite of the fact that both grazing and control bottles contained bacteria, dissolved isotope levels were always higher in grazing bottles (5 to 36 % difference in the fraction of total added isotope remineralized after 9 to 10 h). This strongly suggests that the dominant process driving remineralization in these experiments was zooplankton grazing, although bacteria may have played a role by enhancing release of metals from material already processed by grazers (e.g. fecal pellets, DOC). Microbial release of trace metals from phytoplankton cells and debris has been observed to be a relatively slow process, occurring on a time frame of days to weeks (Frey & Small 1979, Lee & Fisher 1992), even when the microbial community is artificially enriched (Lee & Fisher 1994). Extensive microbial degradation of live plankton cells in the time frame of the crustacean grazing experiments presented here (hours) is unlikely.

A direct comparison of these experiments with those of Lee & Fisher (1994) is difficult because they used different metals, grazers, prey, and incubation times. The results presented here nevertheless suggest a somewhat more important role for grazers in trace metal recycling. An argument can be made that zooplankton grazing is likely to be the dominant process driving metal recycling in the euphotic zone based upon 2 observations:

(1) Many studies have suggested that the fate of most phytoplankton production, in many areas of the ocean, is to be grazed. This has been found to be the case in marine environments ranging from coastal and shelf waters (Laws et al. 1988, Smith & Lane 1988) to open ocean oligotrophic and high-nutrient areas (Tsuda et al. 1989, Frost 1991, Welschmeyer et al. 1991). Microbial degradation of ungrazed cell material certainly contributes to elemental remineralization, and could be especially important during episodic algal blooms when phytoplankton production can greatly exceed grazing rates (Legendre 1990). In most cases, however, the evidence points to grazing as the primary agent in the initial remineralization of planktonic biomass in surface waters.

Additionally, the results of laboratory investigations such as those of Caron et al. (1985), Goldman et al. (1985) and Andersen et al. (1986) suggest that bacteria play a minor role in major nutrient regeneration compared to protozoan grazers. Frey & Small (1979) also concluded that bacterial remineralization of Fe was unlikely to be important compared to grazer-mediated recycling

(2) The acidic conditions which initiate the digestive process in most grazers are exceptionally well suited to solubilizing most trace metals. The pH within copepod guts can fall as low as 5 during digestion (Dall & Mori-

arty 1983), and the pH within the feeding vacuoles of some protozoans may drop to 3 (Fok et al. 1982). The combination of enzymatic degradation and low pH appears to be ideal for returning organically bound cellular metals to inorganic dissolved forms.

Support for this argument comes from the work of Frey & Small (1979), who demonstrated that iron in a cell homogenate became available to phytoplankton only after exposure to a low pH. They hypothesized that grazers may play an important role in iron recycling largely because of their acidic digestion processes.

Iron released from ingested proteins by acidic and enzymatic digestion could even be reduced to the highly soluble ferrous form (Fe^{2+}), depending on redox conditions during the initial stages of digestion. The initial acidic phase of digestion in metazoan and protozoan grazers is usually followed, however, by an alkaline phase prior to egestion (Fok et al. 1982, Dall & Moriarty 1983). Under these conditions, any iron not assimilated by the grazer would likely be quickly oxidized and precipitated as fresh iron oxyhydroxides. In this form, iron can become available for phytoplankton uptake through thermal and photochemical dissolution processes (Rich & Morel 1990, Wells & Mayer 1991).

Increased dissolved concentrations of major nutrients during grazing are the result of inputs from both ingestion and digestion (Jumars et al. 1989). 'Sloppy feeding' or other losses during ingestion may also have contributed to the increases in dissolved trace metal concentrations in these grazing experiments. Because the gut residence time of crustacean grazers is short (i.e. approximately 30 min for copepods; Reinfelder & Fisher 1991), solubilization of egested material may also contribute significantly over the course of these 9 to 10 h experiments.

Jumars et al. (1989) suggested that carbon is rapidly lost from egested fecal pellets because a large proportion is present in soluble forms within the pellet matrix. Fisher et al. (1991) observed a rapid diffusion of Zn, Cd, Am and Pu from copepod fecal pellets, and suggested that a similar 'pore water' loss mechanism might be responsible. The increases in soluble Fe, Mn and Zn observed in the experiments presented here could be partially due to this type of remineralization mechanism, especially if acidic digestive processes have a significant influence on metal speciation prior to egestion.

Experimental conditions in laboratory grazing bottles inevitably differ from those found in the natural environment. It is possible that unnaturally high concentrations of fecal pellets and prey cell and animal surfaces in experimental bottles could lead to an underestimation of the true amount of metal remineralization. Because Fe and Mn (and to a lesser extent

Zn) are highly particle-reactive, scavenging onto artificially dense concentrations of fecal pellets and phytoplankton cells could tend to rapidly reduce dissolved metal levels originating from grazing activity. Nolan et al. (1992) added unlabeled fecal pellets to seawater containing dissolved Co previously released from labeled phytoplankton cells. They observed that the pellets scavenged 50 % of the dissolved Co activity within minutes, supporting the view that metal partitioning in enclosed grazing experiments may not accurately mimic partitioning during grazing in the more dilute environment of the ocean.

In order to deal with the possibility that scavenging of remineralized metals onto surfaces could be biasing results, 10^{-4} M EDTA was added to some of the experiments presented here (Expts 3 & 4; Figs. 3 & 4) to serve as a 'dissolved trap' for the remineralized isotopes. It is possible that this addition of a metal chelator could lead to a bias opposite to that mentioned above, by solubilizing metals originally weakly bound to surface sites associated with fecal pellets. Because of these 2 potentially conflicting artifacts, Expts 3 & 4 (with EDTA) may represent an upper limit to remineralization efficiency, while Expt 1 (without added EDTA; Fig. 1) may represent a lower limit. Thus the 'true' proportion of originally added metals remineralized in this set of experiments probably lies somewhere between 14 and 42 %.

EDTA in seawater is a relatively weak chelator for most metals, because inorganic side reactions of both chelator and metals with major ions tend to greatly lower effective or conditional stability constants. In the surface ocean, the chemical speciation of some metals such as Zn (Bruland 1989) and Cu (Coale & Bruland 1988) is dominated by low concentrations of very strong natural organic chelators. The extent of organic complexation of Fe in the ocean is at present unknown, although some marine organisms are known to be capable of producing very strong chelators known as siderophores (Trick 1989, Reid & Butler 1991). Thus, natural chelators could potentially play an important role in stripping trace metals from fecal pellets in the euphotic zone before their removal by sinking. Such chelation is, however, likely to be by small concentrations of strong natural ligands rather than by high concentrations of weaker ligands such as the EDTA added in these experiments. This scenario is less likely in the case of Mn, since chelation is believed to play only a minor role in Mn speciation in the ocean (Bruland et al. 1991).

Variations in prey and grazer densities and resultant differences in efficiency of food processing and assimilation may account for some of the range observed in proportions of isotopes remineralized during grazing. Another factor which could affect the amount of metal remineralization is the nutritional status of the prey and the grazers. It is known that the extent of major nutrient remineralization by protozoans is a function of the elemental ratios of the prey; for instance, grazers regenerate less nitrogen when fed N-limited phytoplankton than from N-replete cells (Goldman et al. 1987). The experiments presented here made no attempt to compare regeneration efficiencies from metal-limited and metal-replete prey. If trace metal regeneration efficiency is also dependent on prey elemental ratios, prey grown at very low metal:nitrogen ratios might be expected to yield substantially less metal regeneration than the levels obtained here using cells grown at non-limiting metal concentrations.

Rapid, grazer-mediated regeneration of cellular metals to the dissolved phase makes these elements available for uptake over short time periods. Efficient grazer assimilation of elements ingested from prey is another mechanism which tends to extend the residence time of nutrients in surface waters (Fisher et al. 1991). Assimilated elements may later be excreted, making them available to support future production. Incorporation into zooplankton biomass also makes required nutrients available to support secondary production by higher trophic levels.

The amount of metal activity incorporated into the animals at the end of the feeding period in these experiments represented only a fraction of the total added. Amounts in the grazers ranged from slightly over 1 % (Fe in Expt 3) to 19 % (Zn in Expt 4). These percentages do not comprise a majority share of the added activity, although in some cases they do equal or exceed the amounts associated with fecal pellets or are equivalent to dissolved levels.

Efficiencies of Fe assimilation in these experiments ranged from 7 to 22 %, higher than values for several non-required particle-reactive elements measured by Fisher et al. (1991) and Reinfelder & Fisher (1991). They reported typical assimilation efficiencies for elements such as Am and Pu ranging from less than 1 % to a few percent. Values obtained in the present set of experiments for the biologically required, particle-reactive element Fe were about an order of magnitude higher than this, although not as high as values for major nutrient elements such as phosphorus (72 %; Reinfelder & Fisher 1991). Assimilation efficiencies for Zn in this set of experiments ranged from 12 to 56 %; Fisher et al. (1991) reported a value of 48 % for this metal. Mn was least efficiently assimilated in the experiments presented here (2 to 10 %), a range of values only slightly higher than literature values for non-essential particle-reactive metals. Differences in experimental organisms and conditions may account for some of the variability in measured assimilation efficiencies.

Reinfelder & Fisher (1991) reported that assimilation efficiencies from log-phase diatom prey were substantially lower than from stationary-phase prey. Results of the experiments presented here support their observations, in that higher assimilation efficiencies were measured from stationary-phase cultures of diatoms than from log-phase cultures (Table 2). An opposite trend was, however, observed when flagellates were used as prey, in that assimilation efficiencies for individual metals were higher from log-phase prey cells.

It should be noted that growth of both log-phase diatoms and flagellates during the grazing period may have resulted in overestimates of assimilation efficiencies in Expt 3. This growth during the grazing period means that more cells were consumed than was apparent from cell counts, which could result in underestimation of the total activity consumed by the grazers. Since assimilation efficiency is defined as activity retained after depuration over total activity consumed, underestimation of activity consumed would result in overestimation of assimilation efficiency. Additionally, the species assemblage used for the stationary phase grazing experiment was somewhat different ($\frac{1}{3}$ cladocerans, $\frac{2}{3}$ copepods) from that used for the log-phase experiment (all copepods). For these reasons, comparisons between the two should be made cautiously.

These experiments suggest that trace metals can be incorporated into metazoan grazers from both phytoplankton and protozoan prey. The emerging consensus is that protozoans may make up a large fraction of the diet of many larger zooplankton (Stoecker & Capuzzo 1990, Small & Ellis 1992), including *Acartia tonsa* (Stoecker & Egloff 1987), the species which dominated the natural grazer community in several of the experiments presented here. Carnivory by metazoan grazers may represent a significant route through which trace metals can be transferred from the microbial loop into higher trophic levels.

These experiments also suggest that protozoan grazers could be important remineralizers of trace metals from picoplankton prey, although because of potential filtration artifacts further research will be necessary to confirm these preliminary results. Protozoans are believed to be the major consumers and regenerators of primary and bacterial production in many oceanic regimes (Caron & Goldman 1990). Microheterotrophs could potentially play an important role in the biological cycling of trace metals in these regions, as is the case for cycling of major nutrients.

CONCLUSIONS

Remineralization of Fe, Mn and Zn from living plankton cells can be significantly enhanced by the grazing activities of zooplankton. These results suggest that the biota may play an important role in the marine biogeochemistry of these elements. Future work will, however, be necessary to determine the relative importance of grazing and bacteria in trace element remineralization, and to quantitatively examine the role of grazers in metal cycling in natural environments. It seems likely that most trace metals may be involved in cycles of regenerated biological productivity in the euphotic zone, despite the fact that the cycling of particle-reactive elements such as Fe and Mn has usually been assumed to be dominated by abiotic factors. It is becoming increasingly apparent that biologically required trace metals must be considered to be nutrients, with all of the various reciprocal interactions between marine biology and chemistry that this classification implies.

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