Regeneration, recycling, and trophic transfer of trace metals by microbial food-web organisms in the pelagic surface waters of Lake Erie

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Abstract

Rapid regeneration of ^{109}Cd and ^{65}Zn from their picoplankton prey into the dissolved phase by microzooplankton was observed in water sampled from the pelagic surface waters of Lake Erie (summer 1994 and 1995). Trace metals were added to grazing (lake water <210 μm) and control (lake water <0.2 μm) treatments in the form of radiolabeled *Synechococcus*. Picoplankton (0.2–3 μm) were grazed heavily by consumers in the nanoplankton (3–20 μm) and microplankton (20–210 μm) size classes (collectively referred to as microzooplankton) as confirmed by dilution assays used to independently measure grazing activity. Most consumed trace metals were regenerated into the dissolved phase (<0.2 μm), but some trophic transfer of ^{109}Cd and ^{65}Zn from radiolabeled prey into the nanoplankton and microplankton did occur: ^{65}Zn was transferred 2.5 times more efficiently into the microplankton and 2.9 times more efficiently into the nanoplankton than was ^{109}Cd. Recycling of regenerated ^{109}Cd back into plankton biomass was greater than that for ^{65}Zn. Grazing by microzooplankton influenced the molecular size distribution of regenerated trace metal in the dissolved phase (77±6% ^{109}Cd <5,000 MW; 8±24% ^{65}Zn <5,000 MW). These results show that microzooplankton grazing tends to prolong the residence times of metals such as Cd and Zn in the pelagic surface waters of large lakes.

The ultimate geochemical fate of particle-reactive trace metals in pelagic zones of lakes is controlled by the vertical flux of metal in the water column, where metal loss is governed by the sinking of particulate matter to the underlying sediment (Fig. 1A). Metals may become associated with this particulate matter by various sorptive processes (scavenging) such as adsorption, co-precipitation, and, in the case of organisms, cellular internalization. Various inorganic surfaces, such as calcite, clays, and iron and manganese oxyhydroxides, may be involved in scavenging, but in the pelagic environment, biological surfaces are considered to dominate scavenging processes (Sigg 1994; Murray 1987; Morel and Hudson 1985). This is particularly true in the pelagic surface waters of the Laurentian Great Lakes of North America, which are effectively isolated from sediment influences during thermal stratification (Eadie and Robbins 1987).

Among the biological particles, picoplankton (bacteria, cyanobacteria, and algae; 0.2–2 μm) are ideally suited to the scavenging of trace metals (Fisher 1985) owing to their rapid growth rates and high surface area-to-volume ratios.

Picoplankton are heavily grazed by a group of heterotrophic and mixotrophic organisms in the nanoplankton (2–20 μm) and microplankton (20–200 μm) size classes, collectively referred to as the microzooplankton (mixotrophic and heterotrophic nanoflagellates, dinoflagellates, ciliates, rotifers, and crustacean nauplii). Because the ecological fate of picoplankton in the Laurentian Great Lakes is largely determined by microzooplankton grazing (Fahnenstiel et al. 1986, 1991*a*), it follows that trace metals scavenged by picoplankton will also be affected by this activity (Fig. 1B).

In a study of water sampled from the equatorial Pacific Ocean, Hutchins et al. (1993) suggested that microzooplankton regenerated iron from Fe-radiolabeled picoplanktonic cyanobacteria into the dissolved phase and thereby increased bioavailability of iron to larger phytoplankton. Indeed, in a model food chain in the laboratory, the grazing activity of microzooplankton has recently been shown to regenerate trace metals (^{137}Cs, ^{109}Cd, ^{65}Zn, ^{153}Gd) from radiolabeled picocyanobacterial prey into dissolved forms (Twiss and Campbell 1995). The simplified microbial food web studied by Twiss and Campbell involved two microorganisms that represent major contributors to the microbial food web of the Laurentian Great Lakes (Fahnenstiel et al. 1991*a*), namely the mixotrophic nanoflagellate *Ochromonas* grazing on the picocyanobacterium *Synechococcus*. Trace metal regeneration was tightly coupled to grazing activity. We extended this hypothesis of trace metal regeneration by microzooplankton into the field in order to test the influence of the microbial food web on trace metal fates. Radiolabeled picoplanktonic cyanobacteria (*Synechococcus leopoliensis*) were added to sampled lake water containing the natural microbial community found in Lake Erie during summer-stratified conditions. We assessed the effect of biotic activity on the partitioning of the trace metal radionuclides ^{109}Cd and ^{65}Zn, as well as ^{137}Cs, among

A.

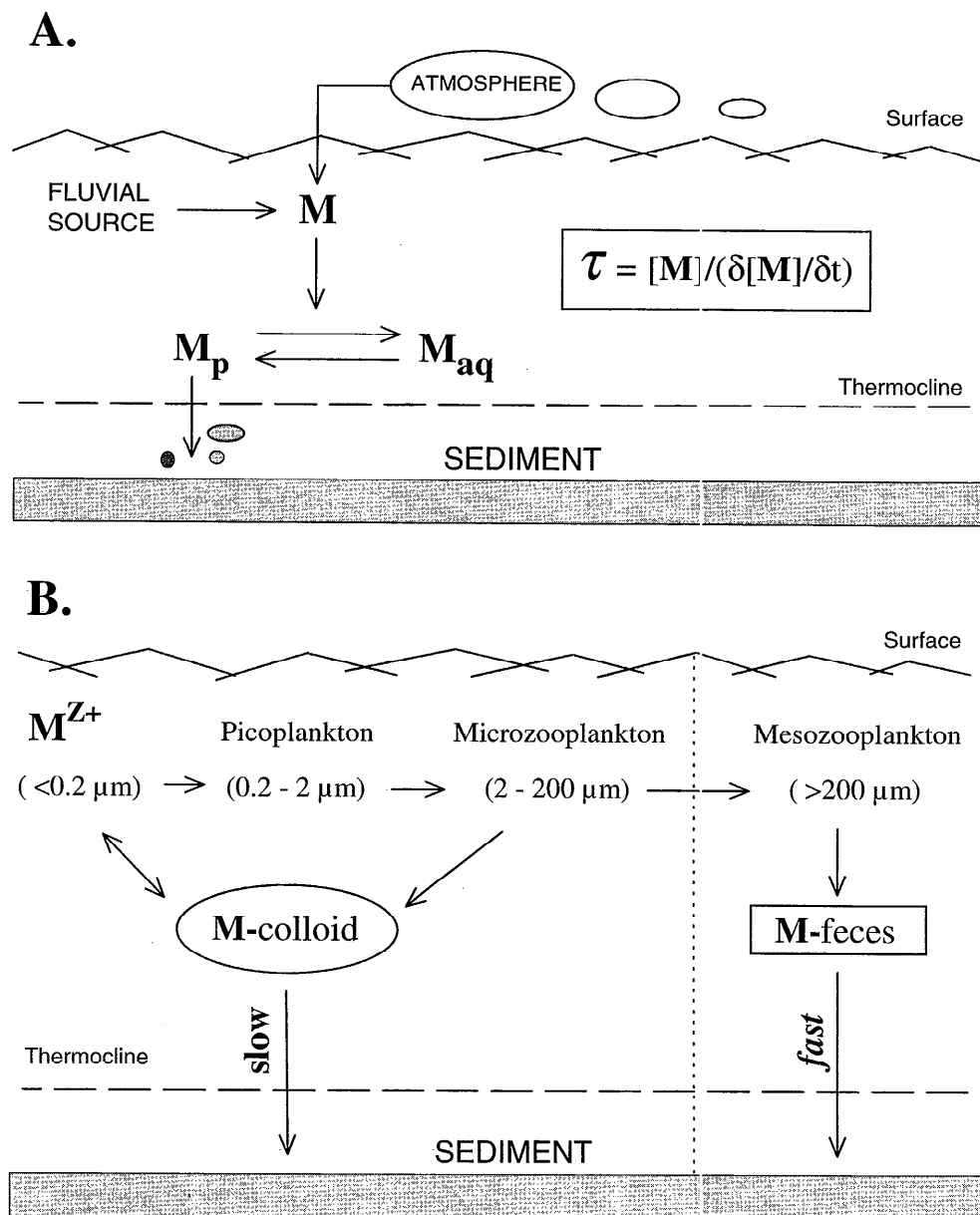$$\tau = [M]/(\delta[M]/\delta t)$$

B.

Fig. 1. The biogeochemical fate of trace metals in the pelagic region of large lakes during thermal stratification. A. Physical and chemical influences. Trace metals enter the water column in particulate or dissolved form and partition among dissolved (M_{aq}) and particulate (M_p) phases. The flux of particulate metal to the sediment controls the residence time (τ) of trace metals in the surface waters. B. Biological influences. Most particles in pelagic regions are autochthonous; among the most productive of these particles are the picoplanktonic algae and bacteria, which have a high potential to scavenge trace metals from the dissolved phase. The ecological fate of the picoplankton is consumption by microzooplankton, the group of mixotrophic and heterotrophic grazers in the nanoplankton (2–20 μm) and microplankton (20–200 μm) size fractions. In turn, microzooplankton are grazed heavily by mesozooplankton, e.g. crustacean zooplankton >200 μm (Carrick et al. 1991). Any trace metals defecated as fecal pellets from the mesozooplankton will sink rapidly to the sediment. In contrast, egested trace metals resulting from microzooplankton grazing are often complexed in colloidal form and hence are less prone to both sinking and resorption by particles. This study deals with the microbial processes (shown to the left of the vertical dotted line) that influence trace metal partitioning in the water column.
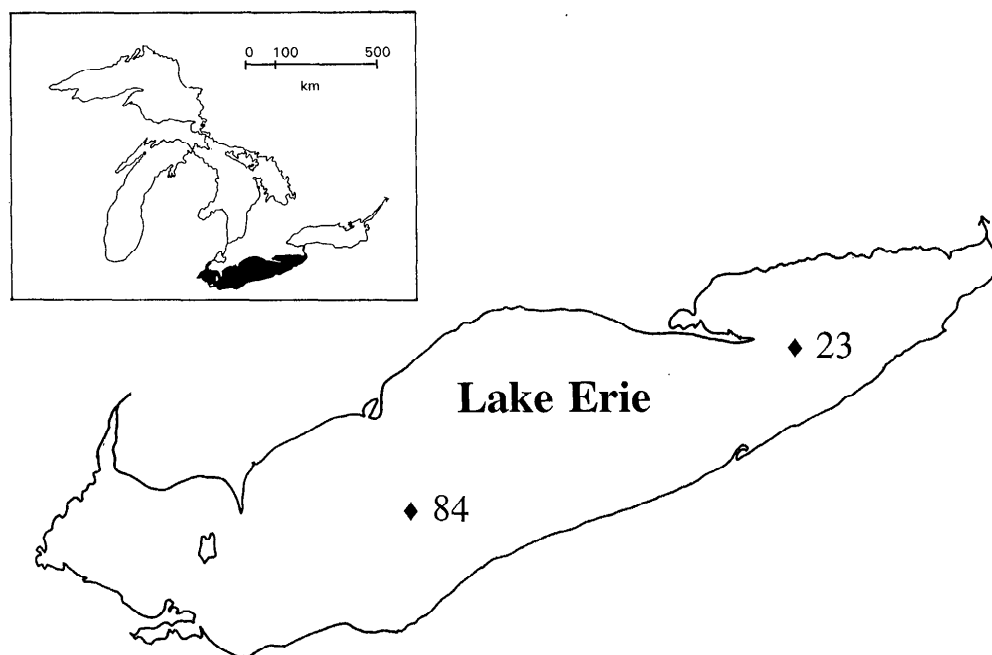
Fig. 2.   Map of the Laurentian Great Lakes showing the position of the study sites in the pelagic zone of the central and eastern basins of Lake Erie. Note: Sta. 23 is also known as Sta. 879.

various particle size fractions and the dissolved phase and showed how geochemical cycles of trace metals and ecological cycles of plankton are linked in pelagic surface waters.

Methods

Glassware preparation—All plasticware and silanized glassware were rigorously cleaned. The cleaning protocol involved a warm soap wash (Liqui-Nox, 1%), methanol soak (HPLC grade), HNO_3 soak (1.6 M, reagent grade), HCl soak (0.8 M; Suprapur, Merck), and sevenfold rinse with deionized water (≥ 17.5 Mohms cm^{-1}) after each cleaning step.

Preexposure of picoplankton to trace metal radionuclides—A culture of the picocyanobacterium *S. leopoliensis* (UTEX 625) (referred to herein as *Synechococcus*) was exposed to the γ-emitting radionuclides ^{137}Cs, ^{109}Cd, and ^{65}Zn in modified Fraquil medium (rFRAt) at a cell density of $\sim 10^{10}$ cells liter^{-1} (Twiss and Campbell 1995). Nominal metal concentrations (and radioactivities) were ^{137}Cs, 5 nM (0.7 MBq liter^{-1}); ^{109}Cd, 5 nM (2.1 MBq liter^{-1}); and ^{65}Zn, 10 nM (10.6 MBq liter^{-1}). After 40–60 h of exposure, these picoplankton were treated with 10^{-4} M Na$_2$EDTA by adding an EDTA solution directly into the rFRAt. After 25 min, the cells were harvested onto 0.4-μm polycarbonate membrane filters (Nuclepore), rinsed with sterile lake water (*see below*), and resuspended. This EDTA rinse technique has proven effective in removing loosely adsorbed trace metal radionuclides from the surface of *Synechococcus* (Twiss and

Campbell 1995). The radiolabeled picoplankton were then used to spike the natural plankton community present in sampled lake water.

Fate of radiolabeled picoplankton in the pelagic microbial community—Forty liters of lake water from the thermally stratified pelagic zone of Lake Erie were collected from a depth of 5 m at Sta. 23 (Fig. 2) at 1830 hours on 11 July 1994 with an acid-cleaned, Teflon-coated 8-liter Go-Flo bottle (General Oceanics) suspended on a nonmetallic line. Lake water was filtered through a 210-μm pore-size polypropylene mesh (Spectrum); this water is referred to as "whole-lake water." Half of the whole-lake water was further filtered (0.2 μm) through an acid-washed (0.1 M HCl; SupraPur, Merck), high-volume membrane filter (Suporcap 100, Gelman Sciences) using an applied pressure of 35 kPa provided by pressurized, HEPA-filtered (Gelman Sciences) prepurified nitrogen gas; this water is referred to as "sterile lake water." All manipulations of collected lake water were conducted in a Class 100 portable clean room fixed to the deck of the CSS *Limnos*. Trace metal analysis of the sterile lake water prepared at Sta. 84 was conducted by G. Lawson. Low concentrations of total dissolved trace metals were present: 2.1 nM Zn, 39 pM Cd, 7.2 nM Cu, 1.7 nM Cr, 21 nM Fe, and 2 nM Mn. These concentrations are comparable to measurements made in Lake Erie surface waters by researchers who used similar trace metal clean techniques (Coale and Flegal 1989; Nriagu et al. 1993, 1996).

Filtration efficiency of the Suporcap membrane filter was verified by comparing the fluorescence of the filtrate (<0.2 μm), after passing $\sim$40 liters of whole-lake water, to the fluorescence properties of the whole-lake water

(Carlson and Shapiro 1981). In vivo fluorescence of whole-lake water was 0.2 fluorescence units (FU), that of filtered whole-lake water (<0.2 μm, polycarbonate membrane filtration of 50 ml) was 0.05 FU, and that of the Suporcap filtrate was only 0.03 FU. Phytoplankton were thus very efficiently removed by the filter.

All water was retained in 20-liter polycarbonate carboys before being dispensed into test bottles through Tygon tubing with pressurized N_2. Experimental treatments were established by adding 2 liters of water to 2-liter polycarbonate bottles (Nalgcne): the grazing treatment received whole-lake water and the control treatment received sterile lake water. The whole-lakc water contained natural microplankton, nanoplankton, and picoplankton communities; the sterile lake water was used as a control for measuring possible desorptive losses of radionuclides from the *Synechococcus* inoculum. The EDTA-rinsed radioactive *Synechococcus* were added to each bottle to give 5.8×10^7 cells liter^{-1}. Bottles were incubated in an incubation chamber to simulate in situ environmental conditions (20°C, photon flux of 92 μmol m^{-2} s^{-1}, and a natural photoperiod). Each treatment was conducted in triplicate.

The fate of the trace metal radionuclides added initially as radiolabeled picoplankton (0.2–3 μm) was determined by serial size-differential filtration at intervals during 51 h. Partitioning of radionuclides was determined among the microplankton (20–210 μm), nanoplankton (3–20 μm), picoplankton (0.2–3 μm), and dissolved (<0.2 μm) fractions. A 100-ml sample from each bottle was removed and gravity-filtered through a 20-μm screen (Nitex, 47 mm), and the filtrate (<20 μm) was removed. The filter was then rinsed with 10 ml of sterile lake water and removed for counting the retained radioactivity. Similarly, 70 ml of the <20-μm filtrate was filtered onto a 3-μm polycarbonate filter (Nuclepore, 47 mm) and, subsequently, 20 ml of the <3-μm filtrate was filtcrcd onto a 0.2-μm polycarbonate filter (Nuclepore, 47 mm). In each case, the filtrate was removed before the filters were rinsed. Applied vacuum was <13 kPa for filtration by the 3-μm and 0.2-μm filters. The final filtrate (<0.2 μm) was sampled (2 ml) in duplicate for radioactivity. Additionally, duplicate 2-ml samples were removed at each sampling interval to determine total aqueous radioactivity in each bottle. The total volume of water removed from the sample bottles by the end of the incubation was <20%.

Assays for measuring grazing rates and growth rates within the microbial community—Phytoplankton-specific grazing and growth rates were assessed by the dilution assay technique (Landry and Hassett 1982) on the same water samples as used in the grazing experiments. Whole-lake water was diluted with sterile lake water to give the following dilution factors: 1, 0.7, 0.5, 0.3, and 0.2. Nitrogen (NO$_3$-N) and phosphorus (PO$_4$-P) were added in Redfield proportions to each bottle (final concentrations of 1 μM and 65 nM, respectively) to control for any effect of regenerated macronutrients on intrinsic growth rates. Dilutions were established in 2-liter polycarbonate bottles and incubated along with the radioactive treatments (*see*

above). After 24 h, aliquots from each bottle were size fractionated by serial filtration and analyzed for chlorophyll *a* (Chl *a*) content.

Shortening the microbial food chain—The purpose of this experiment was to assess the relative roles of nanoplanktonic and microplanktonic grazers in the fate of radiolabeled picoplankton. Water was collected at 1330 hours on 12 July 1994 from a depth of 5 m at Sta. 84 (Fig. 2). A portion of the whole-lake water sample was filtered through a 20-μm screen (Nitex); lake water thus prepared contained only the picoplankton and nanoplankton size fractions. Three duplicate treatments were established: control (sterile lake water, <0.2 μm), lake water <20 μm, and whole-lake water <210 μm. Radiolabeled *Synechococcus* were prepared (*see above*) and added to each bottle to give an initial density of 1.2×10^8 cells liter^{-1}. Bottles were incubated, with the accompanying dilution assay treatments, under simulated in situ conditions (20°C, photon flux of 48 μmol m^{-2} s^{-1}, and a natural photoperiod), and samples were removed for size-differential sequential filtration after 6, 23, and 49 h.

Trapping regenerated trace metal with EDTA: A direct measurement of regeneration and recycling—Trace metals regenerated from radiolabeled picoplankton by micrograzers partition between the various particulate phases and the dissolved phase. We measured the recycling of trace metals from the dissolved phase back into particles by comparing the accumulation of radionuclides by the various particulate size fractions in grazing treatments in the presence and absence of EDTA, which served as a trap for any regenerated trace metals (Hutchins and Bruland 1994).

This experiment was conducted at Sta. 23 with lake water collected at 1600 hours on 24 July 1995 from a depth of 5 m. All manipulations were identical to those in the earlier experiment at Sta. 23 conducted on 11 July 1994 (as described above), with the exception that Na$_2$EDTA was added to half of the treatments—three control bottles and three grazing treatment bottles contained 2 μM EDTA, and three control bottles and three grazing bottles received no EDTA.

Synechococcus was pretreated in rFRAt with [109]Cd, 1 nM (3.6 MBq liter^{-1}) and [65]Zn, 5 nM (10.7 MBq liter^{-1}) for 57 h, washed with 10^{-4} M EDTA, rinsed with lake water, and then added to each bottle to give an initial cell density of 5.2×10^7 cells-liter^{-1}. Bottles for this experiment and the accompanying dilution assay were incubated together under simulated in situ conditions (20±2°C, light intensity of 150 μmol photons m^{-2} s^{-1}, and a natural photoperiod). Bottles that contained radioactivity were sampled at intervals over a 65-h period. Samples from control bottles that contained only radiolabeled *Synechococcus* were filtered only onto 0.2-μm filters. Grazing treatments were subjected to sequential filtration (as outlined above).

In this experiment, further size fractionation was conducted by ultrafiltration (5,000 MWCO ultrafilters; Ultraspin 8000, Lida Corp.) of the dissolved fraction (<0.2

μm) from each treatment replicate after 38 h. Ultrafiltration devices were pretreated prior to use to minimize adsorptive losses of radionuclides, as described by Twiss and Campbell (1995).

The use of the 3-μm vs. 2-μm separation between the picoplankton and nanoplankton — The commonly accepted separation between the picoplankton and nanoplankton size fractions is a 2-μm particle diameter (Sieburth et al. 1978). We chose to use a 3-μm separation in our experiments with *S. leopoliensis* because this pico-cyanobacterium has an equivalent spherical diameter of 1.3–1.5 μm, and we found that a 3-μm filter was superior to a 2-μm filter in separating this picoplankter from cell suspensions. In experiments on Lake Erie water, we found no difference in plankton size class Chl *a* concentrations whether we used a 2-μm or a 3-μm filter, despite the possibility that some small cryptomonad nanoflagellates might have squeezed through a 3-μm filter more readily than through a 2-μm filter (Sieburth et al. 1978; Carrick and Fahnenstiel 1989). We consider the 3-μm separation a reasonable lower limit for the nanoplankton size class in the context of the present study.

Analytical procedures — Chl *a*, corrected for pheopigments, was determined onboard by fluorometric analysis after a 24-h extraction in 90% acetone in the dark at 4°C (Parsons et al. 1984).

Cell densities of *Synechococcus* in the preexposure media and prey control treatments were determined with an electronic particle counter (Coulter Multisizer II; 15-μm orifice) on samples fixed with Lugol's iodine (1.5%).

Radioactivity was determined with a γ-spectrometer (LKB Wallac Compugamma 1282; NaI crystal) equipped with a multi-isotope assay option (UltroTerm software). The radioactivity of [109]Cd and [65]Zn was measured in the energy ranges of 20–38 keV and 990–1,268 keV, respectively. Background radioactivity (lake water or filter blanks) was subtracted from the sample counts. Filter blanks were prepared by refiltering filtrate (<0.2 μm) through replicate 20-μm, 3-μm, and 0.2-μm filters and rinsing the filters as usual. Filtrate used for this purpose was collected at the end of the experiment (>45 h). Propagated errors determined from the error of the sample and background counts were <10% ($P < 0.05$).

Low ^{137}Cs radioactivity in samples required precise counting with a Ge(Li) detector (Canberra Instr.) at 661.6 keV. The limited availability of this instrument constrained counting to only one replicate per treatment (chosen arbitrarily to be the second of each triplicate) of the 1994 experiment conducted at Sta. 23. Counting errors were <25% ($P < 0.05$).

Results and discussion

Regeneration of trace metal radionuclides from the picoplankton size fraction — The natural Lake Erie planktonic community exerted a marked influence on the fate of trace metals, as indicated by the rapid remineralization of radionuclides from the radiolabeled picoplankton in
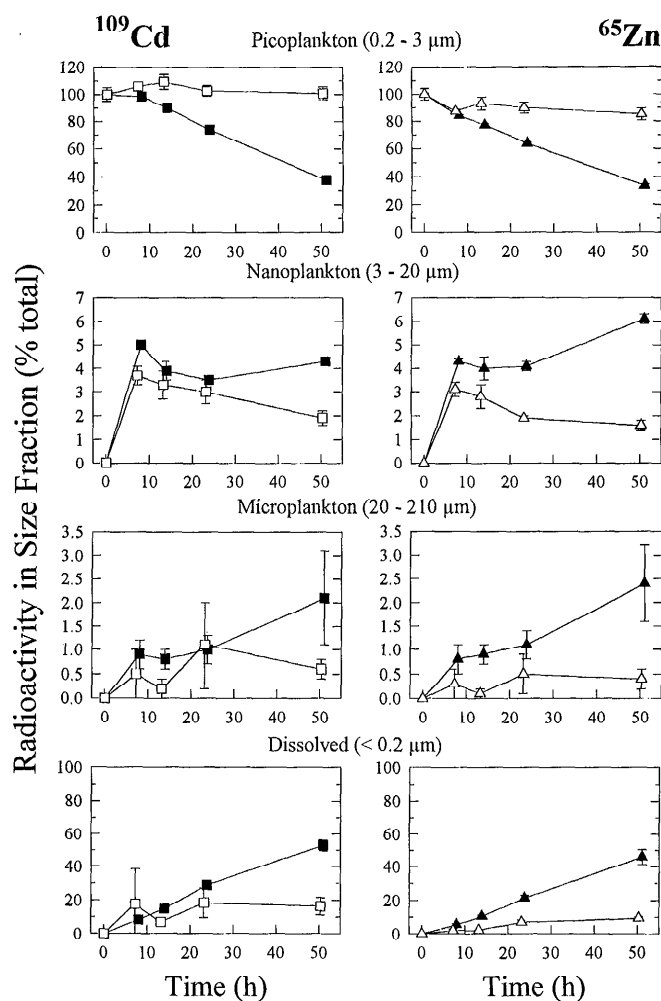

Fig. 3. Partitioning of [109]Cd and [65]Zn among three planktonic size classes and the dissolved phase in grazing (whole-lake water, <210 μm) and control (sterile lake water, <0.2 μm) treatments. Water was sampled from Sta. 23 on 11 July 1994. Radionuclides were added as radiolabeled cells of *Synechococcus* (cell diameter ~1.5 μm) to both treatments. Solid symbols represent grazing treatments; hollow symbols represent prey control treatments. Values are mean ± SD of three replicates per treatment; error bars are shown only when they are larger than the size of the symbol.

the grazing treatments (<210-μm lake water). The disappearance of [109]Cd and [65]Zn from the size fraction that contained the radiolabeled picoplankton spike (0.2–3 μm) was matched by the appearance of these radionuclides in the dissolved (<0.2 μm) phase (Fig. 3). In contrast, the amount of radionuclides desorbing from the radiolabeled *Synechococcus* in the control treatment was markedly lower.

In general, [65]Zn and [109]Cd behaved similarly, with most of the metal having entered the dissolved fraction in the grazing treatments by the end of the experiment (Fig. 3, *see Fig. 6*). For example, partitioning of [65]Zn in the whole-lake water collected from Sta. 23 in July 1994 (Fig. 3) after 51 h was 3% (microplankton), 7% (nanoplankton), 38% (picoplankton), and 52% (dissolved phase) (values
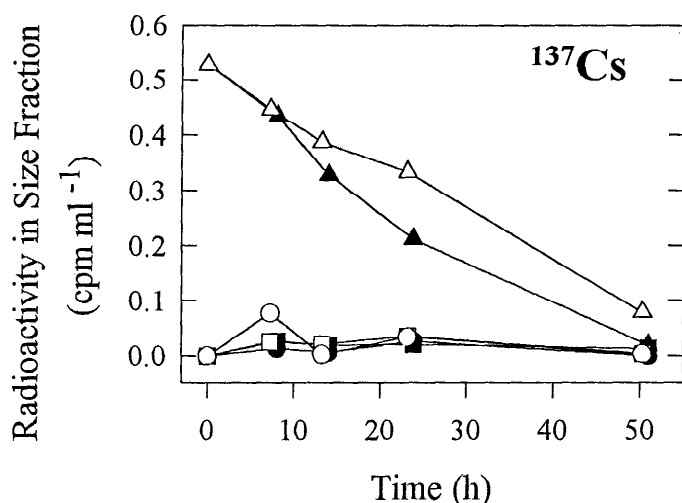
Fig. 4. Partitioning of ^{137}Cs among three planktonic size classes in grazing and control treatments. Water was sampled from Sta. 23 on 11 July 1994. The control treatment (sterile lake water, <0.2 μm) is represented by hollow symbols; the grazing treatment (whole lake water, <210 μm) is represented by solid symbols. Size fractions: picoplankton, 0.2–3 μm (▲, △); nanoplankton, 3–20 μm (■, □); microplankton, 20–210 μm (●, ○). No measurement of ^{137}Cs in the dissolved phase was made. Values are single measurements made from a single treatment replicate.

Table 1. Characterization of the two pelagic study sites in Lake Erie.

Parameter	Station		
	23	23	84
Date	7 Jul	24 Jul	12 Jul
Depth of eplimnion (m)*	15	10	15
Total Chl a (μg liter^{-1})	1.48	1.88	1.65
Microplankton Chl a	0.29	0.59	0.13
(% total)	(20)	(31)	(8)
Nanoplankton Chl a	0.68	0.71	0.93
(% total)	(46)	(38)	(56)
Picoplankton Chl a	0.51	0.58	0.59
(% total)	(35)	(31)	(36)
pH at 5 m	8.1	8.5	8.5
Temp. at 5 m (°C)	21	22	21

* Maximum depth at stations 23 and 84 was 60 and 25 m, respectively.

are percentages of the sum of all fractions). [109]Cd partitioning was virtually identical (2, 4, 39, and 55%, respectively). In contrast, only 10% of the added [65]Zn and 14% of the [109]Cd were found in the dissolved phase in the control treatment. Incidental capture of radiolabeled *Synechococcus* (percent of total radiolabeled *Synechococcus* added) in the control treatment was <0.5% for the 20-μm filter and <1.7% for the 3-μm filter. About 12% of the [65]Zn radioactivity was lost due to filter washes, whereas losses of [109]Cd were not significant, as indicated by the summed radioactivity vs. total aqueous radioactivity measured in each fraction after 51 h (Fig. 3). Mass balances of added trace metal based on changes in the total aqueous radioactivity over the duration of the experiment indicated that there was little loss of radionuclides from sorptive losses to container walls; losses of total radioactivity were generally <5% for [65]Zn and <2% for [109]Cd.

Unlike [109]Cd and [65]Zn, ^{137}Cs was only slightly influenced by the grazing treatment. Very significant losses of ^{137}Cs from the radiolabeled *Synechococcus* were observed in the prey control treatment (Fig. 4). A similar phenomenon was observed in laboratory experiments with the same prey organism in a defined, inorganic growth medium (Twiss and Campbell 1995). Most of the ^{137}Cs loss from the picoplankton cells is attributed to the diffusion of ^{137}Cs from intracellular pools.

Grazing of picoplankton by microzooplankton—Picoplankton comprised 31–36% of the phytoplankton biomass at the stations studied (Table 1). Dilution assays

conducted on the same water samples with no added radiolabeled *Synechococcus* showed high grazing rates of both picoplanktonic and nanoplanktonic Chl a in surface water sampled at both stations (Fig. 5). Repetition of the dilution assay at Sta. 84 on 14 July 1994 and Sta. 23 on 6 September 1994 revealed that grazing of picoplankton was approximately twice that of the nanoplankton or microplankton: picoplankton comprised 48% and 49% of the total Chl a during these sampling times. With the exception of the dilution assay conducted simultaneously with the experiment at Sta. 23 in July 1995, which did not provide a sound estimate for the grazing rate of picoplankton (data not shown), the disappearance of [109]Cd and [65]Zn in the picoplankton size fraction in the grazing treatments (Table 2; Fig. 3, 6) was consistent with the grazing of picoplankton biomass observed in the dilution assays. We conclude that the natural microzooplankton present in the whole-lake water were actively grazing the radiolabeled *Synechococcus* and regenerating the radionuclides into the dissolved phase by their digestive processes.

Relative importance of nanoplanktonic and microplanktonic grazers—Both nanoplanktonic and microplanktonic grazers participated in the grazing of the added radiolabeled *Synechococcus*. Observed net loss rates of picoplankton (i.e. observed loss rate in grazing treatment minus the loss rate observed in prey control) within a given grazing treatment were determined. For lake water with nanoplankton (<20 μm) present, loss rates for [65]Zn and [109]Cd were 0.08 and 0.07 d^{-1}, respectively; for water with nanoplankton and microplankton present, loss rates for [65]Zn and [109]Cd were 0.13 and 0.14 d^{-1}. This comparison indicates that 38 and 50% of the [65]Zn and [109]Cd loss rates from the radiolabeled picoplankton biomass can be attributed to grazing by the microplankton size fraction (20–210 μm). Although studies have suggested that grazers in the nanoplankton size fraction are normally the dominant grazers of picoplanktonic organisms (Fahnenstiel et al. 1991a; Stockner and Porter 1988), the data from this shortened food chain experiment reveal

Table 2. Partitioning of radionuclides after 49 h into various size fractions among different grazing treatments designed to shorten the microbial food chain. Water was collected from Sta. 84. All treatments received radiolabeled *Synechococcus*. Filter-sterilized lake water (<0.2 μm) was used to measure desorption of radionuclides from *Synechococcus*; lake water filtered <20 μm represented indigenous picoplankton and nanoplankton populations, whereas whole-lake water (<210 μm) represented the entire microbial community (i.e. microplankton, nanoplankton, and picoplankton). Values are cpm ml^{-1} ± SD (n = 2); values in parentheses are filter controls. Values in brackets are percentages of the sum of the radioactivity measured in the fractions (this sum does not include radionuclide losses due to filter rinses).

		Lake water fraction		
Radio-nuclide	Size fraction	Sterile (<0.2 μm)	Partially filtered (<20 μm)	Whole (<210 μm)
^{65}Zn	Microplankton	(7±5)	(2±0.4)	3±0.2 [1%]
	Nanoplankton	(4±0.1)	16±4 [5%]	14±1 [4%]
	Picoplankton	256±2 [88%]	210±10 [72%]	191±23 [60%]
	Dissolved	38±3 [11%]	64±2 [20%]	71±17 [22%]
^{109}Cd	Microplankton	(3±3)	(0.3±0.1)	0.4±0.04 [1%]
	Nanoplankton	(1±0.2)	2±0.4 [4%]	2±0.2 [5%]
	Picoplankton	32±0.2 [58%]	27±0.5 [57%]	24±5 [57%]
	Dissolved	19.1±— [36%]	18±1 [38%]	16±2 [38%]

near equal contributions to the grazing pressure on picoplankton by the nanoplanktonic and microplanktonic grazers. In accordance with this finding, Carrick et al. (1992) reported a significant contribution by larger microzooplankton (e.g. ciliates >20 μm) to the grazing control of bacterioplankton in Lake Michigan surface waters during summer months. However, although microplankton were responsible for up to half the grazing impact on the picoplankton in this specific experiment, they accounted for much less accumulation of radionuclides than did the nanoplankton (Table 2). The relatively low ^{65}Zn assimilation efficiency of the microplanktonic grazers (20–210 μm) compared to grazers in the microzooplankton (3–20 μm) may reflect different digestive strategies between organisms in these two groups.

Grazers in the nanoplankton size fraction seemed to regenerate more ^{65}Zn than did those in the microplankton size fraction (Table 2). However, the reduced particle loading in the <20 μm treatment (microplankton comprised 8% of the Chl *a* in the whole-lake water treatment; Table 1) may be responsible for the apparent higher degree of regeneration, since fewer particles would be available to sorb regenerated ^{65}Zn. The results for regeneration of ^{109}Cd into the dissolved phase in the shortened food chain treatments were less clear. An anomalously high
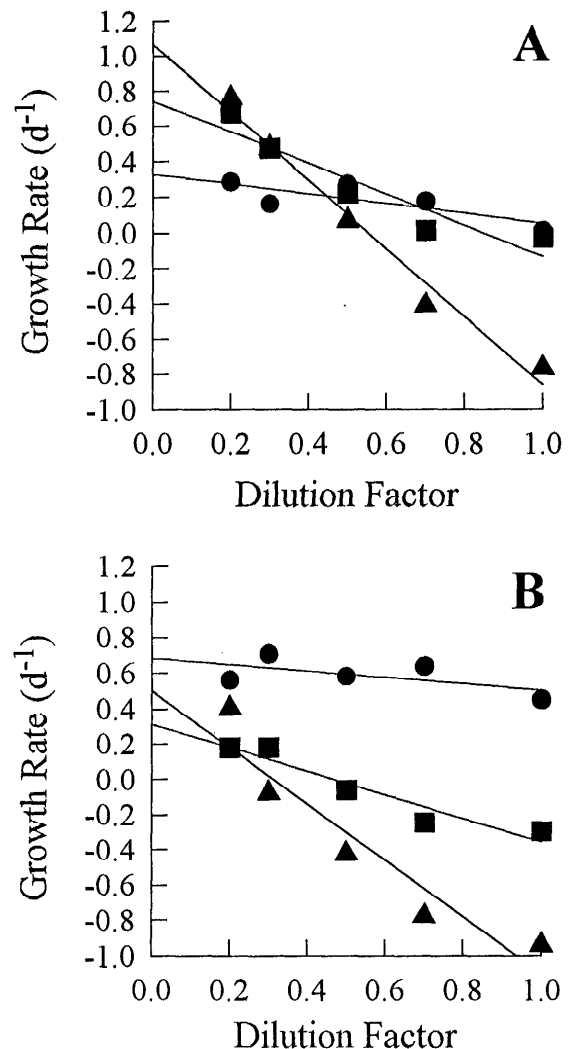

Fig. 5. Results from dilution assays used to estimate the Chl *a*-based specific rates of grazing and growth among the picoplankton (▲), nanoplankton (■), and microplankton (●) size classes from the eastern and central basins of Lake Erie. A. Sta. 23, eastern basin, 11–12 July 1994. B. Station 84, central basin, 12–13 July 1994. Dilution factor is the ratio of whole lake water to sterile lake water in the treatment. Specific growth rates in each dilution were measured in duplicate after 24 h. The slope of the linear least squares regression of growth rate vs. dilution represents the grazing rate, whereas the intercept is the estimated intrinsic growth rate. Observed Chl *a*-based specific growth (μ) and specific grazing (g) rates (d^{-1} ± SE) in each size fraction were: Sta. 23—picoplankton, μ = 1.07±0.10, g = 1.93±0.16; nanoplankton, μ = 0.74±0.12, g = 0.88±0.19; microplankton, μ = 0.33±0.08, g = 0.28±0.12; Sta. 84—picoplankton, μ = 0.50±0.20, g = 1.60±0.32; nanoplankton, μ = 0.31±0.08, g = 0.67±0.12; microplankton, μ = 0.68±0.09, g = 0.18±0.13.

degree of ^{109}Cd loss from *Synechococcus* was observed in the prey control in this experiment (Table 2), effectively masking any regenerative processes. Some desorbed ^{109}Cd may have been readsorbed by particles in the grazing treatments, thereby giving no indication of any ^{109}Cd entering the dissolved phase by regenerative processes. A
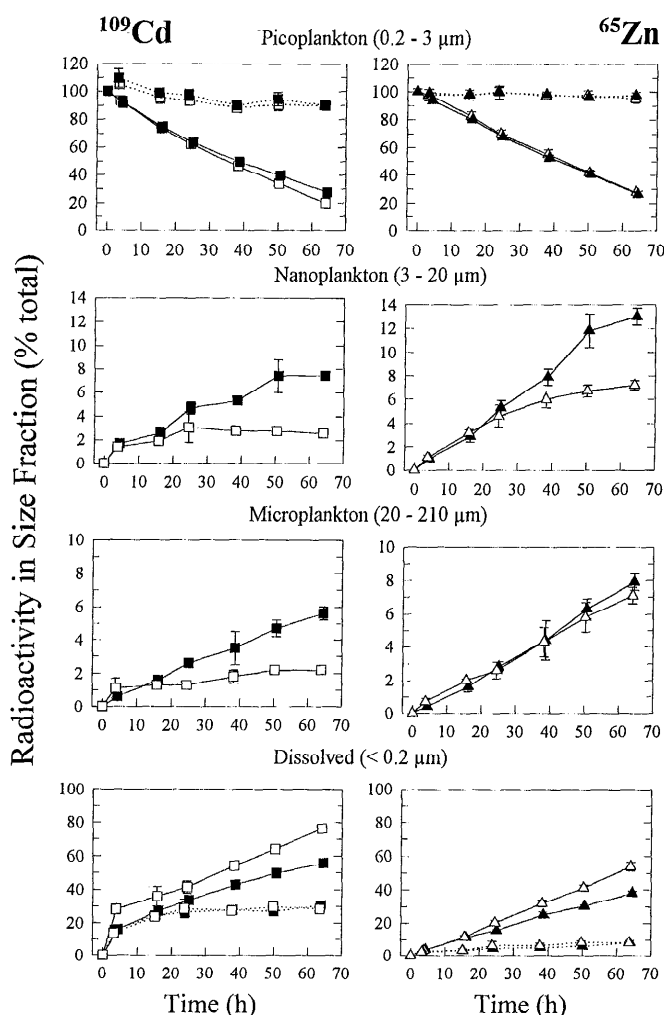
Fig. 6. Partitioning of [109]Cd and [65]Zn among three planktonic size classes and the dissolved phase in grazing (whole lake water, <210 μm) and control (sterile lake water, <0.2 μm) treatments with and without added 2 μM EDTA. Water was sampled from Sta. 23 on 24 July 1995. Radionuclides were added as radiolabeled cells of Synechococcus to all treatments. Solid symbols are for treatments with no added EDTA; hollow symbols represent values for treatments containing 2 μM EDTA. Solid lines represent grazing treatments, dashed lines represent control treatments. Values are mean ± SD of three replicates per treatment (except for [109]Cd in dissolved phase of grazing treatment, $t = 3$ h, $n = 1$); error bars are shown only when they are larger than the size of the symbol.

similar loss of [109]Cd, although less severe, was also observed in the experiment at Sta. 23 (1995; Fig. 6).

Relative importance of trophic transfer and recycling of trace metals within the microbial food web—The transfer of radionuclides from the added radioactive Synechococcus into the nanoplanktonic and microzooplanktonic size fractions was evident in all experiments (Table 2; Figs. 3, 6). In principle, metal movement from Synechococcus into the larger size fractions might occur directly, via trophic transfer, or indirectly, via recycling through the

dissolved phase. Trapping the regenerated trace metals with EDTA allowed us to differentiate between these two routes for [65]Zn and [109]Cd (Fig. 6). Because this experiment was conducted in the presence of excess EDTA, which minimized the scavenging of regenerated trace metals by particle surfaces, accumulation into the nanoplankton and microplankton must have originated from the consumption of radiolabeled prey; in the case of microplankton, the prey may have been both picoplanktonic or nanoplanktonic. The data suggest that trophic transfer from the picoplankton to the nanoplankton and microplankton was more efficient for [65]Zn than for [109]Cd. For example, in the presence of EDTA, [65]Zn was accumulated 2.9 and 2.5 times more than [109]Cd in the microplankton and nanoplankton, respectively. The greater efficiency of [65]Zn trophic transfer may be due to its more favorable intracellular partitioning in the picoplankton prey, compared with [109]Cd (cf. Reinfelder and Fisher 1991). Alternatively, the preference for Zn may reflect the metabolic need for this essential micronutrient in the planktonic community at the time of the experiment (see below).

In the 1994 experiments (see Table 2 and Fig. 3), recycling rates were inferred by comparing the net specific loss rates of radiolabeled Synechococcus in grazing treatments with loss rate of Chl a in the picoplankton size fraction obtained from dilution assays (Fig. 5). The grazing rates obtained from the radioactive grazing treatments were indeed consistently less than those obtained from the dilution assays, but this may have been due to dilution of the natural community by the addition of radiolabeled Synechococcus. Therefore, the rate of radionuclide recycling, i.e. the difference between specific grazing rates obtained using the two techniques, could not be accurately assessed. However, this difficulty was circumvented in the 1995 experiment where the net recycling of regenerated [109]Cd and [65]Zn by the entire plankton in the grazing treatments was estimated as the difference in radionuclide content of size fractions in the presence and absence of added EDTA (Fig. 7A). Because there was no appreciable recycling of the desorbed [109]Cd and [65]Zn by Synechococcus in the prey control treatments, as indicated by the time course of changes in the concentration of radionuclides in the dissolved phase (Fig. 7A, controls) and the picoplankton size fraction (Fig. 7B, controls), this desorbed metal is assumed to be strongly bound by a ligand <5,000 MW (see below), possibly a low-molecular-weight organic complex excreted by the cyanobacterium. Because some of the [109]Cd and [65]Zn lost from the Synechococcus in the grazing treatments was indeed recycled, we conclude that metal originating from the regeneration processes is more bioavailable than that lost in the prey control treatments.

The net recycling of [109]Cd (20% of total radioactivity) was ~20% greater than the net recycling of [65]Zn (16% of total radioactivity), as measured at the end of the experiment (Fig. 7A). At the end of the experiment, most of the recycled Cd was associated with the picoplankton (7.7%, Fig. 7B), followed by the nanoplankton (4.8%, Fig. 7C) and the microplankton (3.4%, Fig. 7D). In contrast, most of the recycled [65]Zn was associated with the na-

noplankton (5.8%, Fig. 7C); the microplankton (1%, Fig. 7D) and picoplankton (~0%, Fig. 7B) were responsible for very little recycling of this regenerated element. The loss of radionuclides (Zn > Cd) attributed to the rinse given to each filter is reflected in the difference between the net recycling measured in the grazing treatments (derived from the level of radionuclides in the dissolved phase at $t = 65$ h; Fig. 7A) and the sum of the recycled trace metal in each specific size fraction after 65 h (Fig. 7B–D; $\Sigma^{109}Cd = 16\%$ and $\Sigma^{65}Zn = 7\%$).

For the microplankton, trophic transfer of ^{65}Zn from radioactive prey was a more dominant source term than was recycling; recycling represented only 11% of the ^{65}Zn accumulated by this plankton fraction after 65 h (Fig. 7D). In contrast, 61% of the ^{109}Cd accumulated by the microplankton was due to recycled trace metal rather than trophic transfer from prey. A similar, yet less pronounced, trend was observed in the nanoplankton after 65 h: accumulation due to recycling was 45% for ^{65}Zn and 64% for ^{109}Cd (Fig. 7C).

The molecular mass distributions of ^{109}Cd ($77\pm6\%$ <5,000 MW) and ^{65}Zn ($8\pm24\%$ <5,000 MW) observed in the dissolved phase after 38 h of the grazing treatment with no added EDTA were similar to those observed when this same picocyanobacterium was fed to a single mixotrophic nanoflagellate in the laboratory (Twiss and Campbell 1995). This parallel between the laboratory and field results suggests that the form of regenerated trace metal may be related more to its cellular localization in the prey than to the digestive strategy of any particular microzooplankter. In comparison, the radionuclide content of the size fraction <5,000 MW was not significantly different from the dissolved (<0.2 μm) content in all the other treatments (prey control treatments with or without EDTA, grazing treatment with EDTA), i.e. 100% ^{109}Cd and ^{65}Zn <5,000 MW. Radionuclides may be regenerated as various organic complexes that presumably reflect the localization of these metals in the prey item. The essential element Zn may be present as metallic cofactor in various enzymes, such as the abundant enzyme carbonic anhydrase (mol wt ~30,000 amu), a contention supported by the size distribution of ^{65}Zn in the dissolved phase. Cd, a moderate substitute for Zn in carbonic anhydrase (Morel et al. 1994), may be co-excreted with this and other Zn proteins, or may be complexed as low-molecular-weight compounds, such as citrate or phytochelatins.

Nanomolar concentrations of high-affinity organic ligands have been found to dominate the speciation of dissolved bioactive trace metals (Zn and Cd) in pelagic marine environments (*see* Bruland et al. 1991). The presence of similar Cu-binding ligands in marine (Moffett 1995) and freshwater environments (Xue and Sigg 1993) is positively correlated with seasonal phytoplankton abundance. It is possible that natural organic ligands that have a greater affinity for Zn and Cd than for EDTA are present in the pelagic surface waters of Lake Erie during thermal stratification. Such ligands, if present at sufficient concentrations, would play a similar dominant role in controlling Zn and Cd speciation in this environment.

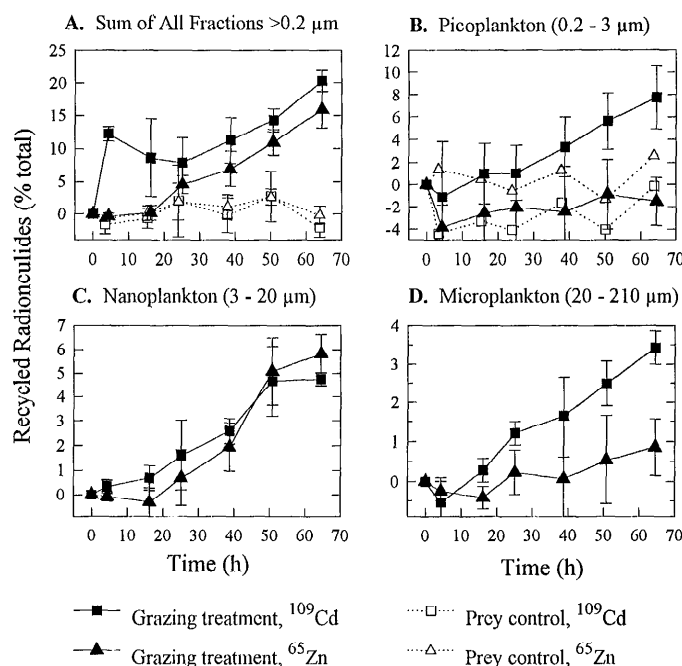In the experiment that used EDTA to trap regenerated

Fig. 7. Estimated recycling of ^{109}Cd and ^{65}Zn from the dissolved phase by various plankton fractions. Recycling was calculated by the difference in radionuclide content between similar size fractions in the presence and absence of 2 μM EDTA (*see* Fig. 6 for details*). A. Net recycling of radionuclides by all particles >0.2 μm in the prey control and grazing treatments. B. As in panel A, except for the picoplankton size fraction (for clarity, bars indicating SD for controls are not shown; controls were not significantly different from zero). C. Recycling of radionuclides into the nanoplankton size fraction in the grazing treatment. D. As in panel C, except for microplankton. Values are % total radioactivity (mean ± propagated SD, $n = 3$) except for ^{109}Cd (sum of all fractions >0.2 μm, grazing treatment, $t = 3$ h, $n = 1$).

trace metals, the addition of 2 μM EDTA was sufficient to complex all of the ambient trace metals plus the trace metal added as radiolabeled *Synechococcus* (assuming 100% remineralization). For example, measured concentrations of dissolved (<0.2 μm) Zn and Cd at Sta. 84 (5 m) were 2.1 nM Zn and 39 pM Cd, and trace metal additions as radiolabeled *Synechococcus* were 0.07 nM Zn and 4.9 pM Cd. Although it is possible that specific natural organic ligands were competing with EDTA, differences in the amount of metal present in the dissolved phase in the grazing treatments in the presence and absence of EDTA (Fig. 6) suggest that EDTA was indeed complexing some of the regenerated ^{109}Cd and ^{65}Zn and thus prevented its recycling.

The hypothesis of trace metal-limited phytoplankton production in the surface waters of Lake Erie during thermal stratification is plausible. Low dissolved concentrations of dissolved trace metals in the surface water (Coale and Flegal 1989; Nriagu et al. 1993, 1996; this study—*see methods*) coupled with moderate levels of phytoplankton biomass (~1.7 μg Chl *a* liter^{-1}; Table 1), and the possible existence of strong complexing ligands of bioactive trace metals (*see above*), would favor trace metal

limitation of phytoplankton. Evidence for a Zn-limited plankton community in this environment is provided by the dissolved Zn profiles from Lake Erie during summer months, measured recently by Nriagu et al. (1996). They suggested that the severe depletion of Zn is due to biological demand in surface waters that exceeds the loading rates of Zn; the most severely Zn-depleted profiles revealed a significant correlation between dissolved Zn and dissolved Cd, indicating similar biological fates for these elements. The addition of an abundant and available source of Zn (as prey) in the grazing experiments described here (e.g. Fig. 6) may have eliminated the need for organisms to use Cd as a Zn substitute (e.g. Morel et al. 1994). Under such circumstances, selection of Zn in favor of Cd would occur during the digestive process of the microzooplankton. Further study is needed to establish the frequency and duration of trace metal limitation in pelagic Lake Erie plankton.

Synechococcus as a surrogate for tracing the fate of trace metals scavenged by picoplankton — Several assumptions are implicit in our use of radiolabeled picoplankton to study the regeneration, recycling, and trophic transfer of metals: microplankton grazers treat the radiolabeled *Synechococcus* as they do other members of the picoplankton community; adding the radiolabeled spike does not radically alter the picoplankton community density; and metals added in the radiolabeled spike are present in representative forms.

We assume that *Synechococcus* represents the entire autotrophic picoplanktonic community and that the fate of trace metals scavenged by heterotrophic picoplankton may also be inferred from the results obtained here with *Synechococcus*. This organism was chosen because the genus *Synechococcus* represents a significant proportion of the primary production in the Laurentian Great Lakes, and it is heavily preyed upon by microzooplankton (Fahnenstiel et al. 1991a). We have assumed that the added *Synechococcus* were as "tasty" as indigenous picoplankton. In fact, protozoa generally prefer large prey over comparable but smaller food items (Chrzanowski and Simek 1990); hence, the larger sized *Synechococcus* (UTEX 625) used in the experiments described here may have been a more attractive food item than the indigenous picoplankton.

Also inherent in our experimental approach is the assumption that the added organisms did not profoundly alter the total autotrophic picoplankton cell density. The amount of Chl a (% total Chl a) added to the experimental treatments as radiolabeled *Synechococcus* was 22% for the experiment at Sta. 23 (1994), 37% at Sta. 84 (1994), and 18% at Sta. 23 (1995). Although the additions of radioactive picoplankton represented a significant fraction of the total ambient Chl a, these levels are biased by the relatively large size of *S. leopoliensis* (estimated spherical diameter, 1.5 μm; the Chl a quota of *S. leopoliensis* was 5.8×10^{-15} g cell^{-1}). Therefore, the contribution of Chl a by the addition of radiolabeled *S. leopoliensis* represents a somewhat greater perturbation to ambient Chl a levels than does the change in cell numbers.

A maximum autotrophic picoplankton density of 2.9×10^8 cells liter^{-1} in the eastern basin of Lake Erie during summer has been observed (Weisse and Munawar 1989): the spikes of radiolabeled *Synechococcus* used at Sta. 23 in the July experiments thus represented $<20\%$ of the maximum density observed in this region. Similarly, the spike of 1.2×10^8 cells liter^{-1} used in the experiment at Sta. 84 represented $\sim 10\%$ of the combined population of autotrophic and heterotrophic picoplankton that has been observed in these areas (Weisse and Munawar 1989).

The metal content of the radiolabeled *Synechococcus* is comparable to what might be anticipated for picoplankton in Lake Erie based on Redfield-like proportions of Cd and Zn in phytoplankton. The cell quotas of Zn and Cd in the radiolabeled picoplankton used in the grazing experiments were estimated to be $C_{106}:Zn_{0.012}:Cd_{0.005}$ (Sta. 23, 1994), $C_{106}:Zn_{0.004}:Cd_{0.0002}$ (Sta. 84, 1994), and $C_{106}:Zn_{0.002}:Cd_{0.0002}$ (Sta. 23, 1995). These estimates for EDTA-washed *S. leopoliensis* are based on the specific activity of radionuclides, the volume of *S. leopoliensis* (1.8 μm^3 cell^{-1}), and a cellular carbon content of 8.83×10^5 mol C μm^{-3} in the picocyanobacteria (Nagata 1986). These elemental ratios are comparable to those measured for pelagic marine plankton collected in the field ($C_{106}:Zn_{0.002}:Cd_{0.0004}$; Bruland et al. 1991) and neritic marine plankton studied in the laboratory ($C_{106}:Zn_{0.01}:Cd_{0.001}$; Morel and Hudson 1985), whereas the estimated Zn quotas are less than those measured in plankton collected from lakes, $C_{106}:Zn_{0.03}$ (Sigg 1994) and $C_{106}:Zn_{0.034-0.1}$ (Reynolds and Hamilton-Taylor 1992). It is possible, however, that metal quotas in naturally exposed and laboratory-raised picoplankton may be distributed differently among various cellular compartments (e.g. present in the cytosol as complexes with phytochelatins, metallothioneins, proteins, or polyphosphates; bound in particulate form to various cellular structures). We have implicitly assumed that the speciation of these elements in the radiolabeled prey is similar to that found in the natural picoplankton community.

Trace metal regeneration as a consequence of microzooplankton grazing — It is becoming increasingly evident that microzooplankton serve to regenerate macronutrients and trace metals in both marine (Caron and Goldman 1990; Hutchins et al. 1993; Hutchins and Bruland 1994) and freshwater environments (Rothhaupt 1992; Taylor and Lean 1981; Twiss and Campbell 1995; this study). With particular reference to trace metals, Hutchins et al. (1993) added radiolabeled *Synechococcus* sp. ($<5 \mu$m) to water sampled from the equatorial and coastal Pacific Ocean, observed the appearance of radioactive Fe in organisms $>5 \mu$m, and inferred that microzooplankton were regenerating Fe into the dissolved phase. A subsequent laboratory study suggested that the protozoan *Paraphysomonas* could regenerate Fe from *Synechococcus* sp. by grazing activity (Hutchins and Bruland 1994), but final proof was limited by potential filtration artifacts. Twiss and Campbell (1995) fed radiolabeled *Synechococcus* to the protozoan *Ochromonas* — a simplified food

chain based on the microbial food web of the Laurentian Great Lakes. They showed that *Ochromonas* could regenerate a suite of trace metals (^{137}Cs, ^{109}Cd, ^{65}Zn, ^{153}Gd) from its prey into the dissolved phase; cell breakage and leakage of radionuclides during filtration were found to be negligible. The same filtration methodology was used in the present study to confirm that these regenerative processes also occur in the pelagic surface water of Lake Erie. We think that the present study provides the first unequivocal demonstration of trace metal regeneration by microzooplankton grazing in the freshwater environment. Moreover, we have confirmed the intensity of grazing activity by using an independent technique—dilution assays. These results suggest that trace metal regeneration will be a functional response of microbial food web activity in all aquatic environments.

The potential for the microbial food web to affect the physicochemical speciation of trace metals and, hence, their geochemical fate, is great. Fisher's (1985) model of trace metal processing by picoplankton assumed negligible leakage of cell contents during grazing (i.e. no regeneration), negligible assimilation from prey to predator, and the loss of picoplankton biomass from the euphotic zone in the form of sinking fecal pellets. Current understanding of microzooplankton grazing of picoplankton is that significant amounts of metal are released from prey during grazing (Hutchins and Bruland 1994; Twiss and Campbell 1995; this study) and that some metal is transferred directly into the grazer biomass with assimilation efficiencies approaching those observed for macronutrients such as N (Hutchins and Bruland 1995). Fecal pellets with an appreciable sinking rate are produced by some microzooplankton (Buck and Newton 1995; Stoecker 1984). However, smaller organisms such as nanoflagellates are much more abundant and have greater clearance rates (Fahnenstiel et al. 1991*a*) than the larger microzooplanktonic organisms that produce these pellets. The fecal matter resulting from exocytosis by some of these smaller organisms is dissolved or colloidal (Caron and Goldman 1990) and would be expected to have an insignificant sinking rate. Clearly, reexamination of the functional role of microbial food web organisms in the control of trace metal geochemistry in surface waters is needed.

Biotic effects on the seasonality of trace metal cycling in surface waters—Recent advances in the understanding of microbial plankton dynamics in the Laurentian Great Lakes (Fahnenstiel et al. 1986, 1991*a, b*), the reliable measurement of trace metal concentrations in these surface waters (Coale and Flegal 1989; Nriagu et al. 1993, 1996), and the linkages between the two, as evidenced here, strengthen the case for strong biological controls over trace metal geochemistry in the pelagic zones of large lakes and, by extension, in oceanic regions as well.

Seasonal floristic changes in the planktonic communities of large lakes undoubtably have an impact on trace metal fates. Resuspension of fine sediments during the isothermal conditions of winter and autumn mixing increase total trace metal concentrations in surface waters, as observed for radionuclide resuspension in the Lake

Michigan (Robbins and Eadie 1991). Temporal studies in lakes have shown the geochemical fates of Zn and Cd to be controlled by diatoms, which bloom in the spring and sink, thereby drawing these trace metals, with silicate, out of the epilimnion (Balistrieri et al. 1992; Reynolds and Hamilton-Taylor 1992). The planktonic community studied here represents biotic influences on trace metal geochemistry in pelagic surface waters of the lower Great Lakes during thermally stratified conditions. As demonstrated here, the actions of the microbial food web during this time of year serve to retain trace metals in the epilimnion.

The importance of the microbial food web is related to nutrient status—its relative importance to water column productivity is inversely proportional to eutrophication (Weisse 1991). Lake Erie has undergone significant reductions in phosphorus loadings in the past 15 yr (Makarewicz and Bertram 1993), and a planktonic community shift from eutrophic to mesotrophic organisms has occurred (Makarewicz 1993). Such a shift would favor the importance of the microbial food web during summer months and thus affect trace metal cycling.

Conclusions

This study emphasizes the importance of considering plankton in surface waters as more than organic sorbents for trace metals. Picoplanktonic organisms are ideally suited for the scavenging of trace metals, and their subsequent grazing by microzooplankton results in both a trophic transfer of these trace metals and their regeneration into the dissolved phase. The balance among these processes—trophic transfer, regeneration, and recycling of trace metals—as affected by aqueous chemistry and seasonal plankton dynamics may determine the geochemical fates of trace metals in surface waters.

The low concentrations of dissolved particle-reactive and bioactive trace metals observed in surface waters in the lower Laurentian Great Lakes in summer months (Coale and Flegal 1989; Nriagu et al. 1993, 1996) are credited to the loss of biogenic particles from the epilimnion by sedimentation, in accordance with scavenging models based on sorptive loss of solutes to particle surfaces (Santschi et al. 1993). Within this model, there are several important implications of the trace metal regenerative and recycling processes that we now know are inherent in the microbial food web. We speculate that the regenerative processes mediated by the microbial food web organisms, as demonstrated here, serve to counterbalance the loss of required trace elements, such as Zn, from surface waters such that Redfield-like proportions of trace elements in phytoplankton biomass (cf. Sigg 1994) are maintained during the season of intense plankton productivity. Trace metal regeneration by microzooplankton provides a dissolved trace metal pool in surface waters (Fig. 1B) that can serve as a buffer, both chemically, via complexation reactions, and physically, by increasing the settling time of trace metals bound by colloids instead of more rapidly sinking particles.

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