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L'INFLUENCE DE LA CHAÎNE TROPHIQUE MICROBIENNE SUR LE CYCLE DES MÉTAUX TRACES DANS LES LACS: ACCENT SUR LA ZONE PÉLAGIQUE DU LAC ÉRIÉ

THE INFLUENCE OF THE MICROBIAL FOOD WEB ON TRACE METAL CYCLING IN LAKES: AN EMPHASIS ON THE PELAGIC ZONE OF LAKE ERIE

Par

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Thèse

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This study is dedicated to The Spirit of The Big Water.

ABSTRACT

The low concentrations of dissolved trace metals observed in the surface waters of the lower Laurentian Great Lakes of North America during summer months are generally attributed to the sedimentary loss of biogenic particles from the epilimnion, in accordance with established scavenging models based solely upon the sorptive loss of solutes to particle surfaces. The majority of particles in the pelagic systems of these lakes are biotic–among the most productive are the autotrophic picoplankton and bacteria (0.2-2 μm) that have a high potential to scavenge trace metals. The ecological fate of picoplankton in the microbial food web is predominately consumption by microzooplankton (mixotrophic and heterotrophic organisms, 2-200 μm).

The hypothesis that microzooplankton can regenerate significant amounts of trace metal into the dissolved phase through the incomplete assimilation of trace metals from their prey was tested in the laboratory and in the field. Radionuclides were used to follow the fate of trace metals ingested in a particulate form by microzooplankton. Rapid regeneration of trace metals from the particulate to the dissolved phase (<0.2 μm) was observed in a laboratory model of a simplified Great Lakes microbial food web, composed of a mixotrophic nanoflagellate (*Ochromonas*) grazing [^{137}Cs,^{109}Cd,^{65}Zn,^{153}Gd]-radiolabeled picocyanobacteria (*Synechococcus*). Most of the trace metal consumed as prey was regenerated by *Ochromonas*; regenerated ^{153}Gd, ^{65}Zn, and ^{109}Cd had reduced bioavailability, in comparison with inorganic forms of the same elements.

Trace metal regeneration was also observed in the natural plankton community sampled from the pelagic region of Lake Erie during thermal stratification. [^{109}Cd,^{65}Zn]-radiolabeled *Synechococcus* were used to label the picoplankton community in lake water and to trace the effect of natural grazing activity on the size fractionation of these metals. Trophic transfer of radionuclides (Zn > Cd) from prey (0.2-2 μm) to predators in the nanoplankton (2-20 μm) and microplankton (20-210 μm), was observed as was the recycling of regenerated metals back into the various plankton size fractions (Cd > Zn). Most regenerated trace metal radionuclides remained in the dissolved phase.

A dynamic model of trace metal fate in the microbial food web under steady-state conditions was constructed using observed characteristics of the various plankton size fractions in the microbial food web (ability to scavenge ^{137}Cs, ^{109}Cd, ^{65}Zn and ^{153}Gd from the dissolved phase; potential to regenerate these metals back into the dissolved phase; population dynamics). Trace metal residence times of Cs (514 d), Cd (29 d), Zn (32 d), and Gd (66 d) predicted by the model were 46%, 62%, 58% and 84% greater, respectively, than residence times predicted if microzooplankton grazing activity was eliminated from the model simulations.

These results are the first unequivocal demonstration of trace metal regeneration by microzooplankton grazing activity, and illustrate the importance of the microbial food web in determining the geochemical fates of particle-reactive trace metals in the pelagic surface waters of large lakes during thermal stratification.

Michael R. Twiss

Dr. Peter G.C. Campbell, *directeur*

FOREWORD/*AVANT-PROPOS*

In recognition of the university in which this dissertation was conceived and conducted, this thesis has an extended abstract written in French to facilitate the communication of the research described herein to francophone readers.

Chapters 2, 3, 4 and 5 have been written in the style of complete manuscripts. Thus, some repetition of concepts may be detected in the introductions, and some descriptions of analytical methodology are the same among some of these chapters.

En reconnaissance à l'égard de l'université où cette dissertation a été conçue et mise à exécution, cette thèse possède un résumé étendu en français pour faciliter la communication de cette recherche à la communauté francophone. Le résumé comporte des figures et tableaux qui démontrent les principaux résultats, ansi que des references au texte principal où le lecteur trouvera des explications plus détaillées.

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I. *PROBLÉMATIQUE*

Il devient de plus en plus évident que les organismes planctoniques sont des constituants fondamentaux du cycle biogéochimique de plusieurs éléments traces dans les eaux de surfaces. Dans quelque cas, la croissance du phytoplancton peut être limitée par les métaux traces de points de vue nutritif ou toxique. L'avancement des connaissances sur les interactions entre le phytoplancton et les métaux traces dans les Grands Lacs laurentiens de l'Amérique du nord repose sur deux facteurs majeurs: i) le raffinement des procédures impliquées dans la détermination des concentrations, et ii) la découverte de l'importance écologique de la chaîne trophique microbienne. En effet, l'utilisation de techniques "très propres" pour l'échantillonnage et la préparation des échantillons d'eau en vue de l'analyse des métaux a démontré que les teneurs en métaux traces dissous dans l'eau de surface des Grands Lacs sont très faibles pendant l'été (tableau I). Ces faibles concentrations sont attribuables à la sorption des éléments traces sous forme dissoute sur les particules (piégeage) et à la perte subséquente par sédimentation de ces particules. Aussi, notre compréhension de la chaîne trophique microbienne, dans laquelle nous trouvons les organismes du «picoplancton» (0.2-2µm; bactéries, cyanobactéries, algues) qui sont broutés de façon importante par les organismes mixotrophes et hétérotrophes (2-200 µm; le «microzooplancton»), a révélé une activité intense du monde planctonique (Stockner et Porter 1988). Cette chaîne trophique microbienne offre de nouvelles avenues de recherche sur le devenir des particules et leur piégeage de métaux traces dans les eaux de surface.

Cette dissertation vise à mettre en lumière l'influence de la chaîne trophique microbienne sur le devenir des métaux traces dans les Grands Lacs laurentiens. Les Grands Lacs contiennent environ 20% de l'eau douce mondiale et sont donc une richesse naturelle hautement significative à l'échelle du globe. Cependant, ces lacs subissent des stress anthropiques constants–par exemple, le rejet dans ces eaux de

déchets industriels et municipaux. Pour améliorer notre compréhension des cycles géochimiques des métaux traces dans les systèmes aquatiques, nous proposons une hypothèse intégrant les concepts courants de «devenir biologique» et «devenir géochimique» des métaux traces; l'hypothèse est développée pour les Grands Lacs laurentiens, et en principe, elle est applicable également à d'autres grands systèmes aquatiques.

Tableau I. Sommaire des concentrations en métaux traces dosés dans la zone pélagique des Grands Lacs laurentiens comparées à celles mesurées en haute mer. Les eaux ont été échantillonnées et analysées par des techniques minimisant la contamination.

Métal Trace	Zone pélagique des lacs: Supérieur	Érié	Ontario	Océan: Atlantique	Pacifique
Cadmium (pM)		77 ± 14^{a} 13 ± 7^{b} 39^{d}	18 ± 6^{a} 183 ± 132^{c} 44 ± 10^{b}	2^{e}	2.8^{f}
Zinc (nM)	4.52 ± 3.43^{b}	0.63 ± 0.19^{a} 0.37 ± 0.05^{b} 2.10^{d}	0.19 ± 0.07^{a} 2.55 ± 0.76^{c} 2.90 ± 0.76^{b}	0.06^{e}	0.23^{f}

Note: [a] Coale et Flegal 1989; eau de surface, 0-1 m. [b] Nriagu et al. 1996; eau de surface (0-2 m, lac Érié et lac Ontario; 2-21 m, lac Supérieur). [c] Nriagu et al. 1993; eau de surface, 1-10 m. [d] station 84; 5 m (*voir* Section 3.3.3). [e] Bruland et Franks 1983, eau de surface. [f] Bruland et al. 1994, eau de surface.

La distribution des métaux traces dans les lacs – Le destin final pour la plupart des métaux traces qui entrent dans un lac est d'être incorporés dans les sédiments. Il est possible de faire un modèle simple de ce processus où le temps de résidence (τ_M) d'un métal trace susceptible de réagir avec les surfaces des particules dépend des interactions entre les métaux et les particules:

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

où, $[M]$ = la concentration de métal dissous dans la colonne d'eau, et $\delta[M]/\delta t$ = la vitesse de disparition de métal dans le système. Une fois que le métal est piégé par une particule, son devenir devient celui de la particule, c'est-à-dire, que son taux de sédimentation correspond à celui de la

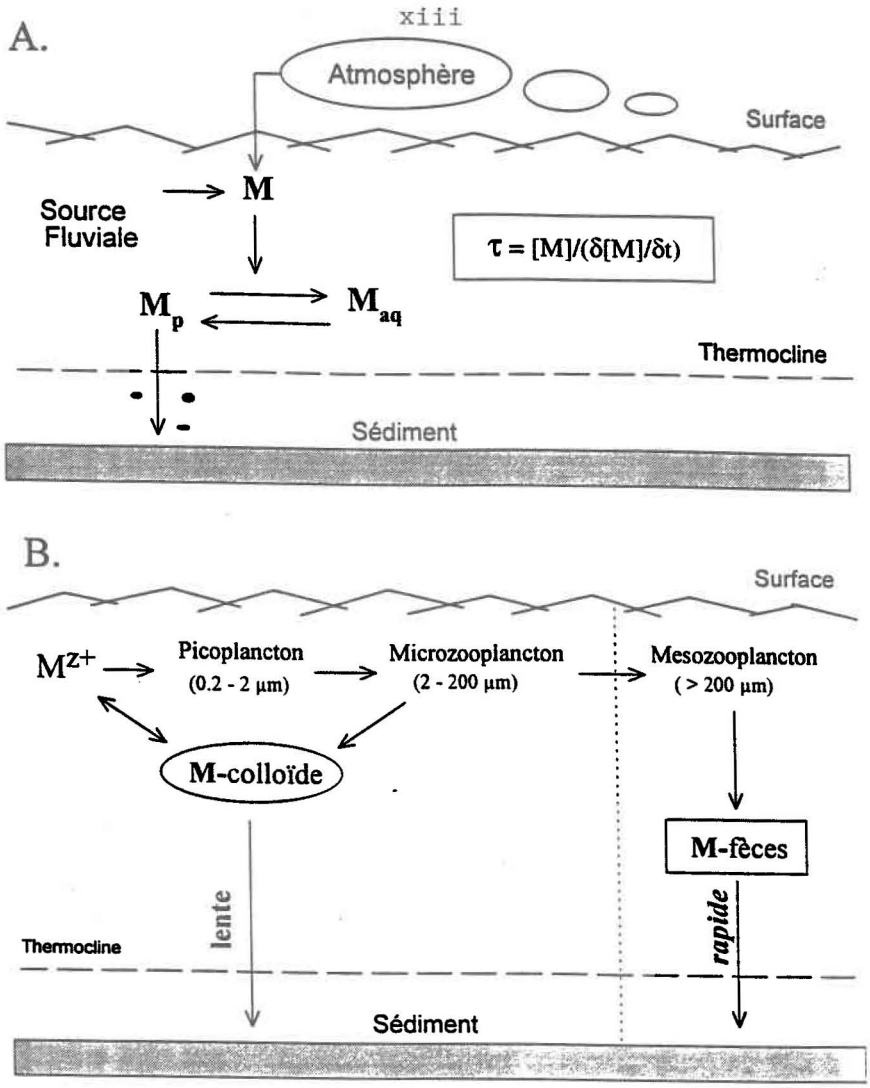

Figure I. Le devenir biogéochimique des métaux traces dans la zone pélagique des Grands Lacs pendant la période de stratification thermique. **A.** Les influences chimiques et physiques. Les métaux traces entrent dans la colonne d'eau sous formes dissoutes ou particulaires; ils se répartissent entre la phase dissoute (M_{aq}) et la phase particulaire (M_p). Le flux de métal au sédiment sous forme particulaire est le facteur qui contrôle le temps de résidence (τ) des métaux traces dans les eaux de surface. **B.** Les influences biologiques. La majorité des particules dans la région pélagique sont autochtones; parmi ces particules, les plus productives sont le picoplancton (algues, cyanobactéries, et bactéries) qui ont un haut potentiel pour piéger les métaux traces dans la phase dissoute. Le devenir écologique du picoplancton est d'être consommé par le microzooplancton, un groupe de brouteurs mixotrophes et hétérotrophes incluant des membres du nanoplancton (2-20 µm) et du microplancton (20-200 µm). Par la suite, le microzooplancton est brouté par le mésozooplancton, ex. les crustacés zooplanctoniques >200 µm (Carrick et al. 1991). Les métaux traces déféqués sous forme de fèces de mésozooplancton sédimenteront rapidement. Par contre, les métaux évacués par le microzooplancton sont souvent sous forme colloïdale et donc, les métaux sont moins susceptibles d'aller au fond et moins disponibles pour les réactions de sorption aux surfaces des particules. Le but de cette étude est de mettre en évidence les activités microbiennes (à la gauche de la ligne verticale pointillée) qui influencent la répartition des métaux traces dans la colonne d'eau.

particule (figure I.A). Cette répartition des métaux traces dans la colonne d'eau, entre les formes particulaires et dissoutes, est considérée comme le facteur clé qui contrôle les temps de résidence relatifs des métaux (Schindler 1989).

Le devenir des métaux traces dans les systèmes aquatiques est influencé significativement par des facteurs physiques, chimiques et biologiques. Contrairement aux petits lacs, l'influence des sédiments sur le cycle des métaux traces dans la colonne d'eau de la zone pélagique des grands lacs, comme les Grands Lacs laurentiens de l'Amérique du nord, est minime pendant la période de stratification thermique. La chimie de l'eau dans les Grands Lacs devrait donc être plus stable que celle dans les lacs plus petits.

Des synthèses récentes sur la distribution des métaux traces dans la mer (Morel et Hudson 1985, Murray 1987, Whitfield et Turner 1987) allèguent effectivement que les influences biotiques sur la chimie des métaux traces dans la colonne d'eau sont très importantes. Par extension aux grands lacs, nous considérons que le biote pourrait jouer un rôle clé dans la détermination des flux de métaux dans la zone pélagique des Grands Lacs. Si la chimie du lac est relativement stable et l'influence des sédiments négligeable, les variables qui influenceront la distribution des métaux traces dans l'épilimnion de la zone pélagique sont: les apports atmosphériques, les processus sédimentaires (flux des particules vers les sédiment), et les processus biologiques. Les processus sédimentaires doivent impliquer le biote (ex.: la sorption des métaux traces par les organismes vivants ou par les détritus biogéniques; la précipitation de la calcite biogénique; la sédimentation de ce matériel d'origine biologique et des fèces qui contiennent des métaux).

À partir d'études faites sur de grands lacs en Suisse, Sigg (1987) a démontré que les métaux traces dans l'eau de ces lacs étaient adsorbés premièrement par la phase particulaire organique. Des mesures chimiques faites sur les particules sédimentaires (captées dans des pièges à sédiment) ont révélé que ces matériaux étaient d'origine autochtone. Selon Balistrieri et al. (1992), qui ont observé une corrélation forte entre les profils du Zn et de la silice réactive dans le lac Sammamish (WA), le devenir du zinc est contrôlé par la biomasse des diatomées; une telle corrélation a été observé au lac Michigan (Shafer and Armstrong 1991) et au lac Windemere (Reynolds et Hamilton-Taylor 1992) pour le contrôle du Zn.

L'écologie du plancton et la chaîne trophique microbienne – La découverte récente (fin de la décennie 1970) de picoplancton photosynthétique (0.2-2 μm) dans les mers et lacs autour du monde a

stimulé beaucoup de recherche pour évaluer l'importance écologique de ce groupe de phytoplancton historiquement négligé (*cf.* Stockner et Antia 1986, Stockner 1991). Présentement, nous savons que ces algues sont très productives et qu'elles sont broutées principalement par de petits organismes mixotrophes et hétérotrophes qui font parti du nanoplancton (2-20 µm) et du microplancton (20-200 µm)[1]. À cause de leurs grands taux de production et de consommation, les picoplanctons autotrophe (cyanobactéries, algues) et hétérotrophe (bactéries) sont considérés importants dans le recyclage de carbone dans la zone photique. Selon le schéma de la «boucle microbienne», le picoplancton autotrophe assimile du carbone inorganique avant d'être consommé rapidement par les consommateurs dans le microzooplancton; dans une certaine mesure, le carbone initialement fixé par le picoplancton est perdu à cause de la respiration du picoplancton et du microzooplancton. À son tour, le microzooplancton est brouté par le zooplancton >200 µm; de nouveau il y a d'autres pertes du carbone dues à la respiration et l'inefficacité de la digestion du zooplancton. Malgré ces pertes, le transfert du carbone demeure encore davantage efficace que si le microzooplancton n'était pas brouté intensément par le zooplancton (c'est-à-dire, si son devenir était contrôlé par lyse cellulaire ou sédimentation, le transfert serait encore moins efficace; Carrick et al. 1991).

Le devenir des métaux traces: le lien entre les devenir écologique et géochimique – Une publication récente par Fahnenstiel et al. (1991a), portant sur l'écologie de la picocyanobactérie *Synechococcus* dans les Grands Lacs laurentiens, a fourni des informations nouvelles sur son rôle biogéochimique possible dans le cycle des métaux traces. Ce picoplancton avait un taux de croissance spécifique *in situ* de 0.1-0.9·j^{-1} et comptait pour approximativement 10% (gamme 1-26%) de la productivité primaire dans les lacs Huron et Michigan (le picoplancton ayant la fluorescence rouge compte pour 1-7% de la productivité dans ces lacs; Fahnenstiel et al. 1991b). En comparaison, Fahnenstiel et al. (1986) ont démontré que 50% de la productivité primaire dans le lac Supérieur pouvait être attribuable à la fraction planctonique <3 µm. Des pertes par broutage se chiffraient à 33-120% du taux de croissance par *Synechococcus*; 68% de ces pertes étaient attribuable à des petits (4-10 µm) nanoflagellés hétérotrophes et ciliés (ex.: *Ochromonas*, *Katablepharis* et *Urotricha*), alors que 5-21%

[1] Les consommateurs dans les taille de plancton de nanoplancton et microplancton sont désignés le microzooplancton; il s'agit d'organismes mixotrophe et hétérotrophe (2-200 µm), ex.: nanoflagéllés hétérotrophes, ciliées, rotifères, dinoflagéllés, nauplii des zooplancton crustacés.

étaient causées par les rotifères et crustacés. En comparaison, le phytoplancton plus gros (ex.: le microplancton autotrophe, 20-200 μm) avec des taux de croissance spécifique plus bas (0.05-0.4 $\cdot j^{-1}$), subit des pertes majeures de population dues à la sédimentation, au broutage par le zooplancton et à l'autolyse des cellules (Scavia et Fahnenstiel 1987).

Les nanoflagellés mixotrophes et hétérotrophes, ainsi que les ciliés, ingèrent leurs proies par phagocytose, ce qui implique que les proies cellulaires sont engouffrées *in toto*. Par exemple, *Ochromonas*, un membre dominant du microzooplancton dans les Grands Lacs laurentiens, possède des flagelles qui jouent un rôle dans la saisie et la sélection des proies; ils peuvent apporter la proie jusqu'à la surface cellulaire (Wetherbee et Andersen 1992). *Ochromonas* consomme sa proie par une extension pseudo-podale, qui enveloppe rapidement (≈1 s) la proie cellulaire; cette dernière entre dans une vacuole primaire de nourriture. La vacuole primaire migre vers la partie antérieure de la cellule, où elle se fusionne avec une vacuole digestive secondaire (Capriuolo 1990). Cet événement peut se produire assez rapidement, de sorte que 6 à 8 cellules de picoplancton peuvent s'accumuler dans la vacuole digestive secondaire. Les conditions chimiques dans cette vacuole subissent un changement de pH (milieu neutre ou alcalin vers conditions acides) pour atteindre une acidité maximale d'environ pH 2. Pendant que la digestion se poursuit, la vacuole devient plus grande, le pH évolue vers les conditions alcalines et la digestion finale se produit. De petites vésicules, qui contiennent les produits de la digestion, se séparent de la vacuole digestive secondaire et sont transportées à différents sites dans le cytoplasme. Le reste des matériaux non-digérés est apporté à la surface cellulaire où il est éjectée (Capriuolo 1990).

Puisque plusieurs organismes du microzooplancton se nourrissent par phagocytose, il s'ensuit que le quota total de métal de chaque proie entre à l'intérieur de ces consommateurs. Les métaux traces dans la biomasse vivante peuvent être conservés efficacement par rapport au carbone, puisqu'ils ne sont évidemment pas respirés. Cependant, la régénération des éléments nutritifs limitants N et P par la boucle microbienne a été démontrée (Goldman et al. 1987, Caron et Goldman 1990), et le même processus est susceptible de se produire dans le cas des métaux.

II. *FORMULATION DE L'HYPOTHÈSE*

À partir des arguments présentés dans les sections précédentes, il s'ensuit que la chaîne trophique peut influencer significativement le devenir des métaux traces dans les grands lacs. À cause de leur taux de croissance très rapide, de leur grand rapport superficie:volume cellulaire, et de la capacité de leur surface cellulaire pour l'adsorption des métaux, les organismes dans la taille du

picoplancton ont un potentiel extraordinaire pour piéger les métaux traces dissous. Suivant le devenir écologique du picoplancton, qui est principalement voué à être consommé par le microzooplancton, les métaux accumulés par le picoplancton doivent entrer dans une fraction particulaire plus grande.

Le microzooplancton peut régénérer les macronutriments comme le phosphore et l'azote. Il faut considérer le phénomène selon lequel le rapport des éléments dans la biomasse suit le rapport de Redfield (Morel et Hudson 1985). De plus, l'efficacité de croissance du microzooplancton doit être inférieur à l'unité. Il s'ensuit que pour un organisme du microzooplancton qui gagne son énergie par la consommation de biomasse, cette action de consommation doit être jumelée à l'évacuation des métaux traces accumulés via ses proies (autrement, l'organisme accumulerait un excès de métaux et l'homéostasie représentée par le rapport de Redfield ne serait pas respectée).

Les métaux régénérés par le microzooplancton pourraient être moins susceptibles d'être piégés que la forme de métal piégée initialement par le plancton. Par conséquent, la régénération des métaux traces par l'activité du broutage de microzooplancton (figure I.B) devrait augmenter le temps de résidence des métaux traces dans la colonne d'eau.

Nous proposons ici une hypothèse expliquant le rôle de la chaîne trophique microbienne dans le cycle de métaux traces dans les eaux de surface des Grands Lacs:

HYPOTHÈSE

> Le microzooplancton peut régénérer les métaux traces dans la phase dissoute à partir des métaux traces ingérés via ses proies, dû à une assimilation incomplète lors de la digestion. Il s'ensuit que l'activité de broutage par le microzooplancton augmentera le temps de résidence des métaux traces piégés via ses proies dans l'eau de surface.[2]

III. *OBJECTIFS*

L'objectif primaire de cette dissertation était de montrer que les organismes de la chaîne trophique microbienne peuvent régénérer les métaux traces suite à leur ingestion et digestion de proies qui contiennent ces éléments. Pour ce faire, une série d'expériences contrôlées, utilisant deux

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[2] Lorsque cette hypothèse a été formulée à la fin de 1991, cette idée était nouvelle–aucune étude précédente dans les domaines de la limnologie ou de l'océanographie n'avait abordé ce domaine de recherche. Des études faites depuis cette date ont proposé que la régénération des métaux traces pourrait être effectuée par les protozoaires marines (*se référer au* Chapter 6 *pour un sommaire de ces études*). Cependant, ces études ne démontrent pas le phénomène de régénération de façon définitive. Les communications scientifiques basées sur la présente dissertation constituent la première démonstration sans équivoque de la régénération de métaux traces par les protozoaires en conditions contrôlées de laboratoire (Twiss and Campbell 1995), et sur le terrain en conditions naturelles (Twiss et al. 1996).

organismes (une proie et un prédateur) de grande importance écologique au sein des Grands Lacs laurentiens, a été réalisée en laboratoire.

Le césium, le cadmium, le zinc, et le gadolinium sont les métaux traces choisis pour cette étude. Ces métaux présentent une grande gamme de rapports entre la charge et le rayon (z^2/r: Cs = 0.6, Cd = 4.1, Zn = 5.4, Gd = 9.6) et en conséquence, une grande gamme de réactivités de surface (Gd > Zn, Cd >> Cs). De plus, ces éléments ont différentes valeurs nutritives: le Zn, un oligo-élément essentiel; le Cd, relativement toxique; et l'absence de rôles nutritifs pour le Cs et le Gd.

Le deuxième objectif était de vérifier si les effets du broutage par le microzooplancton sur la répartition des métaux traces entre les formes dissoutes et solides sont décelables dans l'environnement naturel. Ainsi, une extension du plan expérimental effectué en laboratoire a été réalisée dans la zone pélagique du lac Érié.

Finalement, afin que nous puissions évaluer et prédire l'effet du microozooplancton sur la géochimie des métaux traces dans les Grands Lacs, un modèle du devenir des métaux traces par rapport à l'activité de la chaîne trophique microbienne a été développé, basé sur les comportements du plancton et des métaux traces observés dans la zone pélagique du lac Érié. Le modèle prédit les temps de résidence des métaux traces en présence et en absence de la chaîne trophique microbienne.

Limites de l'envergure de l'investigation – Cette recherche est limitée à la zone pélagique des eaux de surface des grands lacs pendant la période de stratification thermique. Dans les grands lacs, comme les Grands Lacs laurentiens, l'influence des sédiments sur la chimie de l'épilimnion est minime et les particules autochtones constituent donc la phase solide dominante qui piège les métaux traces. Ainsi, les implications de la chaîne trophique microbienne dans le cycle des métaux traces, telles que mises en évidence dans la présente étude, ne seront applicables qu'aux systèmes aquatiques avec des propriétés biologiques et physiques semblables (c.-à-d., à l'eau de surface isolée des sédiments par la stratification, où la composition de la population de particules est dominée par le biote).

Aussi, seulement les organismes de la chaîne trophique microbienne (<210 µm) sont étudiés ici comme facteurs impliqués dans les processus de régénération. Nous ne tenons donc pas compte de l'influence possible de la lyse virale du plancton, ni de la régénération par le broutage effectuée par le mésozooplancton, ni de l'effet que ces facteurs pourraient avoir sur la dynamique de la chaîne trophique microbienne. Nous tentons plutôt de comprendre l'effet de la boucle microbienne sur la dynamique des métaux traces; nous focalisons sur les activités de la chaîne trophique microbienne seulement.

Les métaux choisis (Cs(I), Cd(II), Zn(II), Gd(III)) ne sont pas susceptibles de subir des changements d'état d'oxydation sous les conditions de cette étude, incluant les conditions acides dans les systèmes digestifs du microzooplancton. Ainsi, des changements de biodisponibilité, par exemple, un changement de concentration de l'ion libre d'un métal donné, seront attribués à l'effet de complexation du métal par un ligand, et non à un changement du potentiel d'oxydoréduction qui peut altérer la biodisponibilité d'autres métaux traces (ex.: Ce, Co, Cu, Fe, et Mn) dans les eaux de surface.

IV. *MÉTHODOLOGIE*

Étude en laboratoire: modèle simple d'une chaîne trophique microbienne – L'influence du broutage par le microzooplancton sur le devenir des métaux traces provenant d'une proie a été étudiée en laboratoire. L'approche expérimentale impliquait le marquage des proies cellulaires, la picocyanobactérie *Synechococcus leopoliensis*, avec les radionucléides des métaux traces (^{153}Gd(III), ^{65}Zn(II), ^{109}Cd(II) et ^{137}Cs(I)). Les cellules marquées ont été lavées avec de l'EDTA, pour enlever les métaux labiles de la surface des cellules, et ensuite données au prédateur, le mixotrophe nanoflagéllé *Ochromonas danica*. Des traitements témoins (proie sans brouteur, brouteur sans proie) ont aussi été étudiés. Les expériences ont été réalisées sur une période de 43-49 heures dans un milieu d'eau douce défini. La répartition du métal entre le brouteur, la proie, et la phase dissoute a été déterminée pour différents temps selon une technique de filtration séquentielle (filtres de 3 µm, puis de 0.2 µm). Le filtrat <0.2 µm a enfin été filtré avec un ultrafiltre de 5 kD.

Étude sur le terrain: lac Érié – L'approche employée pour les expériences sur le terrain ressemblait à celle utilisée au laboratoire dans la première phase de l'étude. L'eau de surface a été échantillonnée à une profondeur de 5 m à deux stations dans les bassins est et central du lac Érié (figure II) pendant les étés 1994 et 1995. Toutes les étapes d'échantillonnage et de manipulation de l'eau ont été effectuées avec des techniques «propres» pour éviter la contamination de l'eau par les métaux.

L'eau échantillonnée a été tamisée (210 µm) pour enlever le mésozooplancton avant de recevoir les [^{109}Cd, ^{65}Zn]-*Synechoccocus* pour marquer la communauté picoplanctonique (0.2-3 µm). Les même cyanobactéries-marquées ont été ajoutées aux traitements témoins, qui comprenaient l'eau du lac stérilisée par la filtration (0.2 µm). Une expérience semblable à celle faite au laboratoire a été réalisée, sauf que le traitement se faisait en double; la moitié des traitements ont reçu de l'EDTA (2 µM) pour

piéger les radionucléides régénérés par l'activité du microzooplancton, et pour éviter leur recyclage par le plancton.

La répartition du métal entre les différentes tailles de plancton naturel et la phase dissoute a été déterminée pour différents temps selon une technique de filtration séquentielle (filtres de 20 µm, puis 3 µm, puis 0.2 µm). Le filtrat final (<0.2 µm) a enfin été filtré avec un ultrafiltre de 5 kD.

La dynamique (taux de croissance, taux de broutage) du phytoplancton de taille de microplancton (20-210 µm), nanoplancton (2-20 µm) et picoplancton (0.2-2 µm) a été déterminée par les essais de dilution selon la technique de Landry et Hassett (1982).

Études sur le terrain: le piégeage des métaux traces par le plancton de la chaîne trophique microbienne – La colonne d'eau de chaque station de la figure II a été caractérisée pour vérifier l'état de stratification thermique. L'eau a été échantillonnée à une profondeur de 5 m puis tamisée (210 µm). Un mélange de radionucléides a été ajouté à l'eau pour donner des concentrations en métaux traces beaucoup moindres que leur propre limite de solubilité. L'accumulation dans le temps de ^{153}Gd, ^{65}Zn, ^{109}Cd et ^{137}Cs chez le microplancton (20-210 µm), nanoplancton (2-20 µm), et picoplancton (0.2-2 µm) a été suivie pendant une période inférieure à 30 heures.

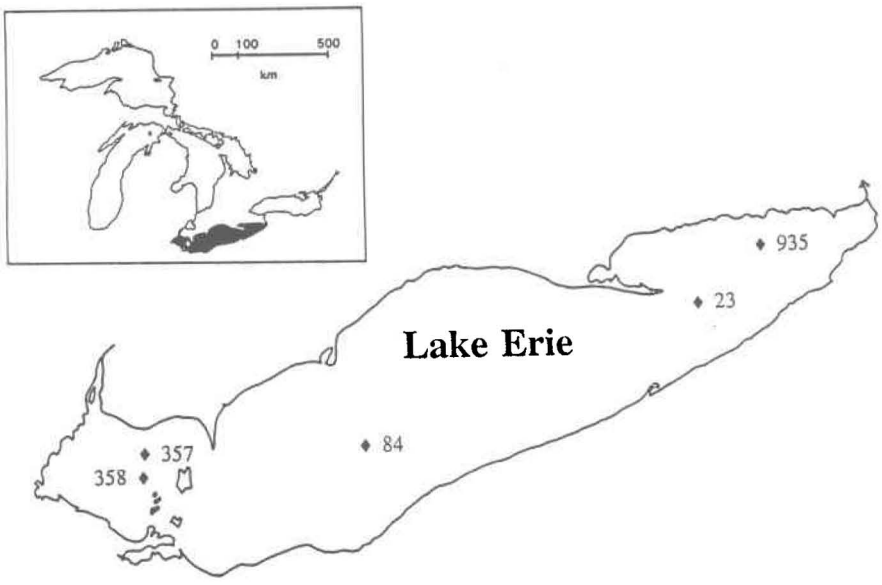

Figure II. Carte des Grands Lacs laurentiens montrant les stations pélagiques du lac Érié.

La modélisation de l'influence de la boucle microbienne sur le cycle des métaux traces dans la zone pélagique du lac Érié – Des informations sur la répartition, la régénération, le recyclage, et le transfert trophique des métaux traces, ainsi que de l'information sur le taux de croissance, les taux de broutage, et la biomasse du picoplancton, du nanoplancton, et du microplancton, de même que leur capacités respectives à piéger les métaux traces dans la phase dissoute, ont été recueillies pour construire un modèle dynamique du devenir de ces métaux dans le monde planctonique de l'épilimnion du lac Érié. La modélisation était basée sur le principe de l'état stationnaire, où le flux d'entrée du métal égale le flux de métal qui sort du système. Avec ce modèle nous avons pu tester l'hypothèse que l'activité de broutage par le microzooplancton augmente le temps de résidence d'un métal.

V. *RÉSULTATS ET LEUR SIGNIFICATION*

Étant donné qu'aucune recherche n'a essayé d'observer la répartition des métaux traces au sein de la communauté planctonique des Grands Lacs laurentiens en fonction de la chaîne trophique microbienne, l'information obtenue ici est très pertinente au plan environnemental.

Étude en laboratoire: modèle simple d'une chaîne trophique microbienne – *Ochromonas* a fortement brouté la picocyanobactérie *Synechococcus* pendant la durée de l'expérience (figure III). Le broutage de *Synechococcus* marqué se manifestait par une apparition rapide des métaux traces dans la phase dissoute (figure IV). Par contre, dans le traitement de témoin (*Synechococcus* seul), la régénération sous forme dissoute du ^{109}Cd et du ^{65}Zn a été mineure. Bien qu'il y ait eu des pertes importantes de ^{153}Gd et de ^{137}Cs par les *Synechococcus* marqués dans le traitement témoin, la régénération de ces radionucléides a été plus élevée dans le traitement de broutage. La majorité des métaux consommés ont été régénérés dans la phase dissoute–nous avons d'ailleurs testé que ces métaux provenaient effectivement du processus d'exocytose et qu'ils n'étaient pas des artefacts de filtration (*se référer à* Appendix B).

Le fractionnement de la phase dissoute par ultrafiltration a révélé que 77% du ^{109}Cd, 25% du ^{65}Zn, 100% du ^{137}Cs, et 74% du ^{153}Gd ont passé dans la fraction inférieure à 5 kD. Le Gd, le Zn et le Cd présents dans la phase dissoute (<0.2 μm) après régénération étaient moins disponibles pour le piégeage par le plancton que dans les milieux synthétiques où ils étaient prisents sous forme purement inorganique. Ces résultats suggèrent que le broutage peut augmenter le temps de résidence des métaux traces dans la colonne d'eau, dû à son effet sur la spéciation des métaux régénérés.

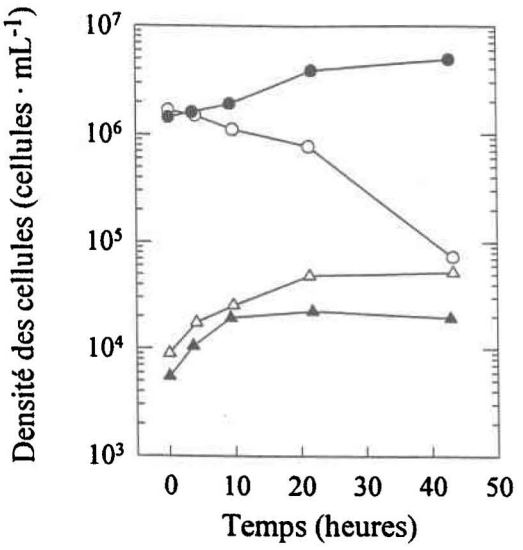

Figure III. La croissance de *Synechococcus* et *Ochromonas* dans les traitements expérimentaux d'une chaîne trophique microbienne simplifiée. Symboles: ●–*Synechococcus* seul; ○–*Synechococcus* avec *Ochromonas*; ▲–*Ochromonas* seul; Δ –*Ochromonas* avec *Synechococcus*.

Étude sur le terrain: vérification du processus de régénération en nature – Comme lors des expériences en laboratoire menées sur une simple chaîne trophique microbienne, nous avons observé une régénération rapide du ^{109}Cd et du ^{65}Zn, à partir des proies picoplanctoniques vers la phase dissoute, provoquée par l'activité du microzooplancton de l'eau pélagique du lac Érié (figure V). L'activité de broutage a été confirmée avec une technique indépendante: les essais de dilution. Le picoplancton (0.2-3 µm), le nanoplancton (3-20 µm), et le microplancton (20-210 µm) étaient broutés par les consommateurs du microzooplancton (3-210 µm). La majorité des métaux traces consommés ont été régénérés dans la phase dissoute (<0.2 µm), mais une partie du ^{109}Cd et du ^{65}Zn, provenant de proies radio-marquées, a été transférée vers le nanoplancton et microplancton par broutage direct (c.-à-d., par transfert trophique). Le transfert du ^{65}Zn a été 2.9 et 2.5 fois plus efficace que celui du ^{109}Cd, dans les transferts trophiques du picoplancton vers le microplancton et le nanoplancton, respectivement. Le recyclage du ^{109}Cd de la phase dissoute vers la phase planctonique à été plus élevé que pour le ^{65}Zn. Le broutage par le microzooplancton a influencé la taille effective des métaux régénérés (77% ^{109}Cd <5 kD, 8% ^{65}Zn <5 kD). Ces résultats démontrent que l'activité de broutage par le microzooplancton peut prolonger le temps de résidence des métaux comme le Cd et le Zn dans la zone pélagique des grands lacs.

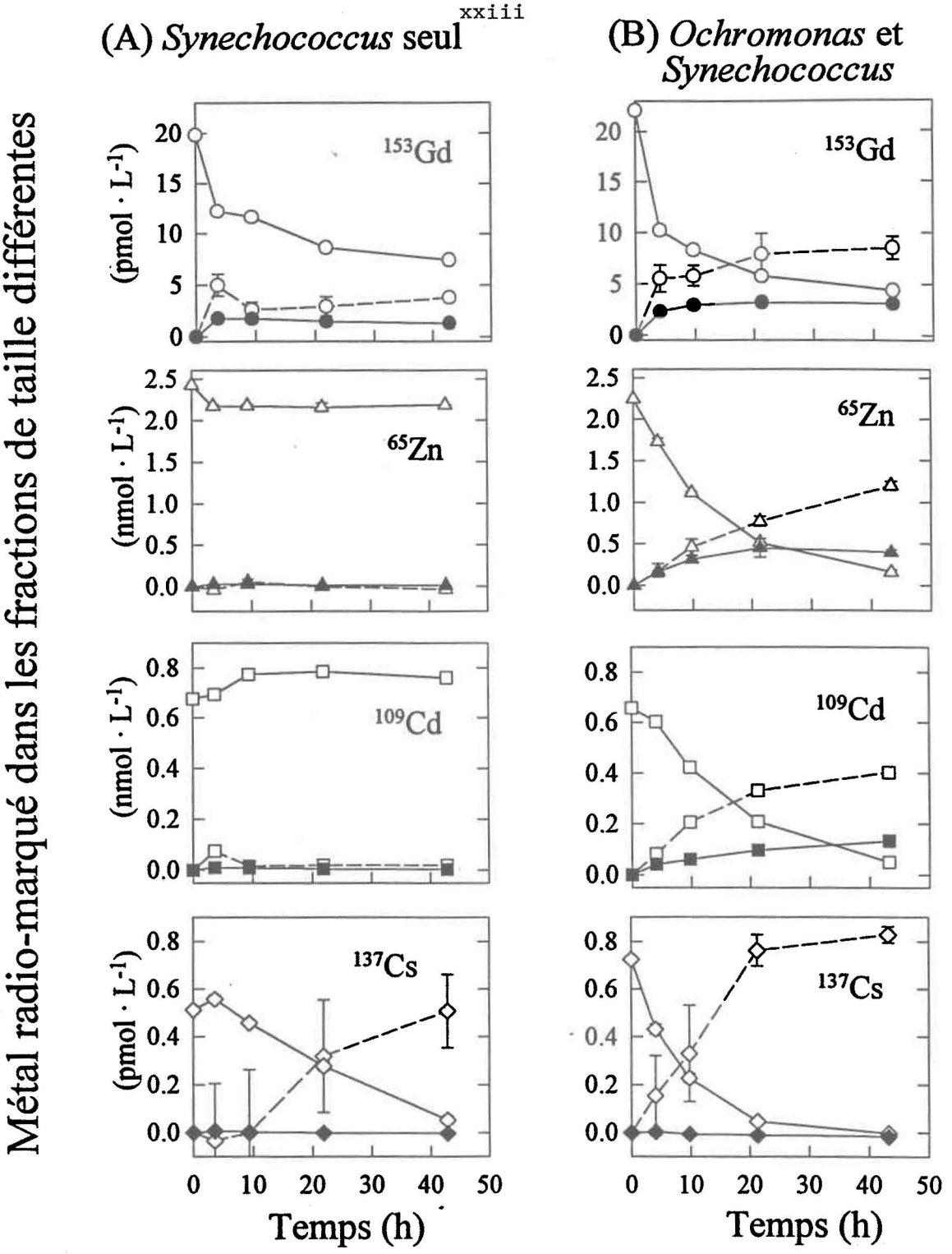

Figure IV. La répartition des radionucléides entre les particules de deux tailles (>3 µm, 3-0.2 µm), et la phase dissoute (<0.2 µm) par l'action d'une simple chaîne trophique microbienne. Les radionucléides ont été ajoutés via les *Synechococcus* marqués (3-0.2 µm) au traitement témoin (*Synechococcus* seul) et au traitement de broutage (*Synechococcus* et *Ochromonas* ensemble). Fractions: >3 µm (symboles pleins); 0.2-3 µm (symboles vides avec ligne pleine); <0.2 µm (symboles vides avec ligne pointillée). Les valeurs sont des moyenne ± ÉT, $n = 3$.

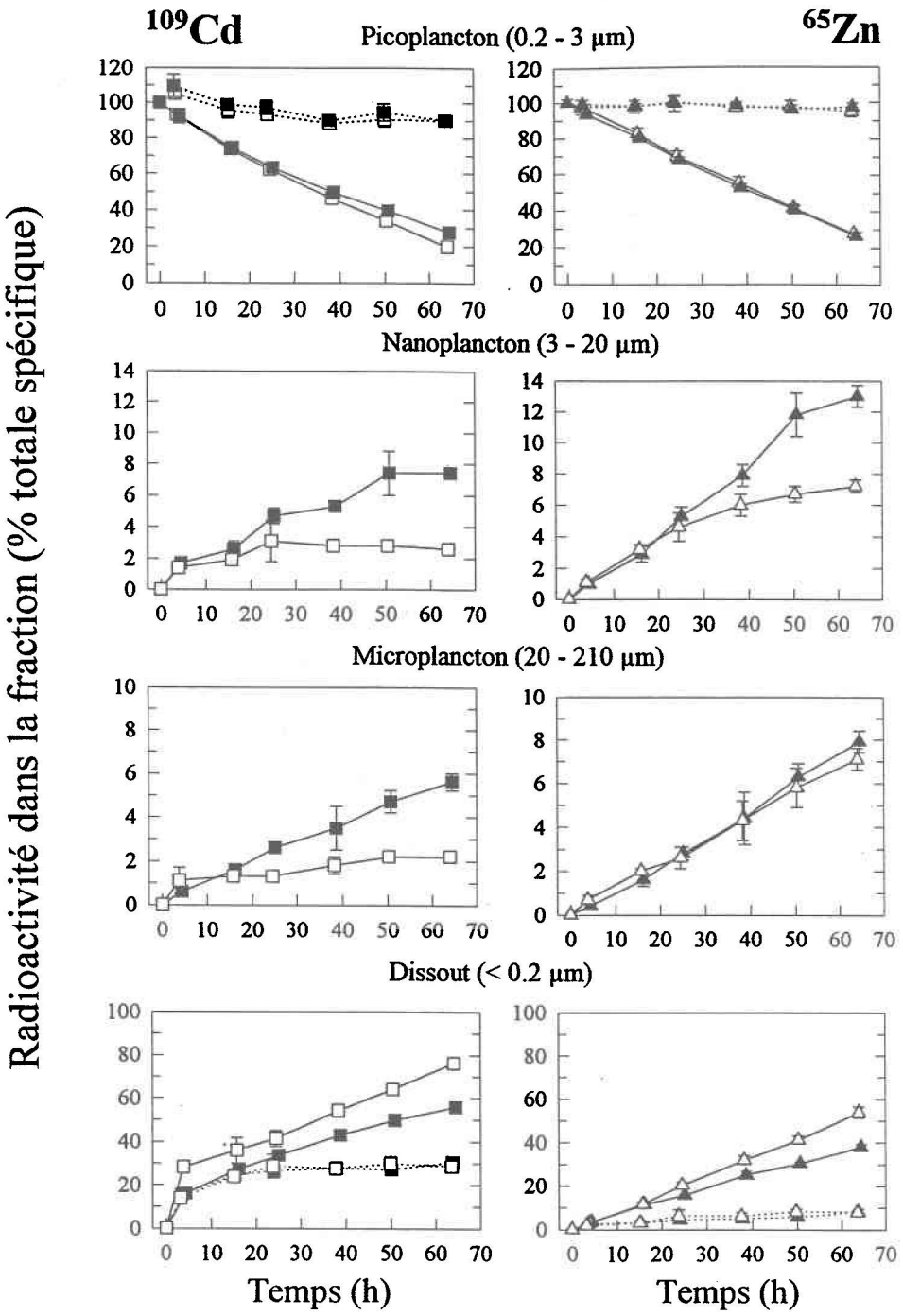

Figure V. La répartition du ^{109}Cd et du ^{65}Zn entre trois tailles de plancton et la phase dissoute dans un traitement de broutage (l'eau de lac filtrée, <210 µm) et traitement témoin (l'eau de lac filtrée, <0.2 µm), en présence et absence de l'EDTA (2 µM). L'eau provenait de la station 23 (24 juillet 1995). Les radionucléides ont été ajoutés à chacun des traitements sous forme de cellules de *Synechococcus* marquées. Les symboles pleins représentent les traitements sans EDTA; les symboles vides représentent les traitements qui contiennent 2 µM EDTA. Les lignes pleines réfèrent aux traitements de broutage; les lignes pointillées sont pour les traitements témoins. Les valeurs sont des moyennes ± ÉT de trois replicats par traitement.

Études du piégeage des métaux traces par le plancton du lac Érié – La détermination du devenir du ^{137}Cs, du ^{109}Cd, du ^{65}Zn et du ^{153}Gd, ajoutés en faibles concentrations à l'eau prélevée de la zone pélagique de lac Érié (<210 μm), avait pour but de tester deux hypothèses: i) le piégeage des métaux traces est effectué par toutes les tailles de plancton comprises dans la chaîne trophique microbienne (picoplancton, 0.2-2 μm; nanoplancton, 2-20 μm; microplancton, 20-210 μm); et ii) le piégeage des métaux est directement relié à la réactivité de chaque métal pour les surfaces des particules (Gd > Zn, Cd >> Cs).

La filtration sélective par taille de plancton, effectuée à des temps successifs jusqu'à 22-30 heures, a démontré que le picoplancton et le nanoplancton sont les pièges dominants pour les métaux traces dans l'eau de surface de lac Érié pendant la période de stratification thermique (figure V). Le piégeage du ^{153}Gd, du ^{65}Zn et du ^{109}Cd par le plancton était plus semblable que prévu selon l'hypothèse de réactivité aux surfaces. Il n'y avait qu'un faible piégeage du ^{137}Cs, sauf aux sites du bassin ouest de lac Érié où l'eau de surface était en contact intime avec le sédiment (source d'argiles). Le ^{65}Zn était l'élément le plus piégé par toutes les classes de plancton, sauf le picoplancton (pour lequel le ^{109}Cd était le radionucléide le plus piégé). Cette accumulation élevée du ^{109}Cd par le picoplancton peut être due à la sorption de cet élément sur la calcite biogénique qui est associée au picoplancton autotrophe. Ces expériences fournissent de nouvelles informations concernant la répartition des métaux traces chez le plancton de la chaîne trophique microbienne (0.2-210 μm)–une communauté dynamique au sein des particules en suspension dans la zone pélagique du lac Érié pendant la stratification thermique. Les résultats suggèrent qu'il faut tenir compte de la dynamique du plancton pour prédire le devenir géochimique des métaux traces dans cet environnement.

La modélisation: liens entre les devenirs géochimiques et écologiques des métaux traces – La perte du plancton des eaux de surface vers les sédiments est considérée comme le facteur clé dans le contrôle des concentrations de métaux traces dans ces eaux. Le picoplancton, qui est très productif dans la chaîne trophique microbienne, est idéal pour piéger les métaux traces et donc influencer leur devenir géochimique. L'hypothèse voulant que la chaîne trophique microbienne puisse influencer le devenir des métaux traces de manière significative a été testée avec un modèle dynamique. Le modèle a estimé les temps de résidence des métaux traces (τ) en supposant l'état stationnaire (flux de métal à l'entré = flux de métal à la sortie): τ_{Cs} = 514 jours, τ_{Cd} = 30 ± 33 jours, τ_{Zn} = 34 ± 22 jours, τ_{Gd} = 71 ± 45 jours (moyenne ± ÉT; valeurs corrigées pour le temps de résidence de l'eau dans lac Érié). Les temps de

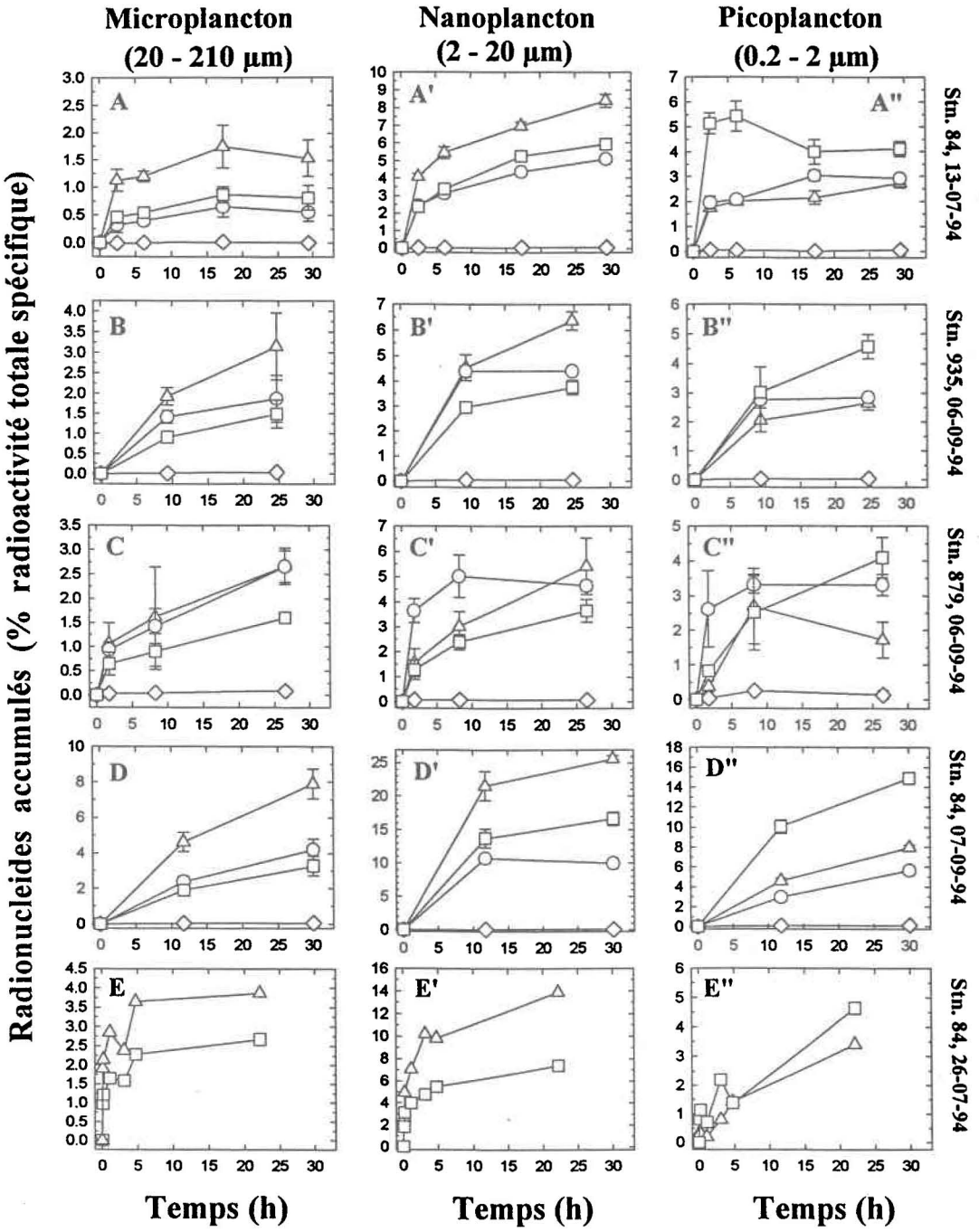

Figure VI. Le piégeage des radionucléides de métaux traces par le plancton de tailles différentes aux stations des bassins est et central du lac Érié. Les sites de la zone pélagique suivent un gradient de biomasse phytoplanctonique croissant (*se référer au* Table 4.1, p. 70). Les valeurs représentent des moyennes ± ÉT ($n = 3$, sauf «E-E"» où $n = 1$). Les métaux traces ont été ajoutés sous forme inorganique dissoute à chaque traitement au début de chaque exposition. Les concentrations totales des métaux ajoutés étaient: Gd, 300-317 pM; Zn, 44-58 pM; Cd, 0.01-179 pM; Cs, 0.1-5 nM: O – ^{153}Gd; Δ – ^{65}Zn; □ – ^{109}Cd; ◊ – ^{137}Cs.

résidence prédits par le modèle sont plus longs que ceux prédits lorsque nous enlevons du modèle l'activité de broutage par le microzooplancton (ex.: Cs, 46% plus long; Cd, 62% plus long; Zn, 58% plus long; Gd, 84% plus long). La prolongation des temps de résidence par le microzooplancton est donc attribuée à la régénération des métaux traces suite à l'assimilation incomplète de ces métaux par les consommateurs. La simulation d'une augmentation de l'abondance de la biomasse picoplanctonique dans la zone pélagique du lac Érié indique des augmentations des temps de résidences pour le Zn et le Gd, mais non pour le Cd.

En comparaison avec d'autres modèles prédisant le temps de résidence des métaux traces dans le lac Érié, le modèle dynamique a prédit des temps comparables au modèle de plancton de type statique (*se référer au* Chapter Five) mais plus courts qu'un modèle de bilan de masse (tableau II). Néanmoins, le modèle dynamique a prédit des concentrations de Cd (4-25 pM) et de Zn (210-850 pM) qui se situent dans la gamme des concentrations dosées par d'autres chercheurs (tableau I). Étant donné que le modèle dynamique est basé sur une base de données indépendante des mesures de concentrations des métaux, la concordance entre les concentrations mesurées et les prédictions par le modèle est encourageante.

Ces résultats mettent en évidence l'importance de la chaîne trophique microbienne en déterminant le devenir géochimique des métaux traces dans la zone pélagique des grands lacs pendant la stratification thermique.

Tableau II. Résumé des temps de résidence des métaux traces dans l'épilimnion du lac Érié estimés par divers modèles d'état stationnaire. Les valeurs du temps de résidence (τ_M) sont corrigées pour le temps de résidence de l'eau dans le lac Érié (τ_W = 986 jours): $\tau_M^{-1} = \tau_W^{-1} + \tau_S^{-1}$, ou τ_S = le temps de résidence d'un métal dû à la sédimentation (prédit par les modèles statique et dynamique).

		Temps de résidence du métal trace (jours)			
Modèle		**Cs**	**Cd**	**Zn**	**Gd**
I.	Plancton statique	510 - 760	13 - 41	10 - 28	4-8
II.	Plancton dynamique	514	30 ± 3	34 ± 22	71 ± 45
III.	Bilan de masse données de:				
Coale et Flegal 1989		---	230	94	---
Nriagu et al. 1996		---	87	180	---

CHAPTER ONE

INTRODUCTION

It is becoming increasingly evident that planktonic organisms are fundamental components of the biogeochemical cycle of many trace elements in surface waters. In some cases, the growth of plankton may be limited by trace metals from either a nutritive or toxic standpoint. Changes to our current understanding of trace metal interactions with plankton in the Laurentian Great Lakes of North America have been stimulated by two major factors: i) advances in trace metal sampling and analysis, and ii) the discovery of the ecological significance of the microbial food web. First, the application of clean sampling and processing techniques has demonstrated that very low total dissolved concentrations of trace metals prevail in surface waters of the Great Lakes during summer stratified conditions (Table 1.1). These low concentrations of trace metals are attributed to the sorption of these elements from solution by particles (scavenging) and the subsequent sedimentary loss of these particles. Secondly, our comprehension of the microbial food web, wherein rapidly growing picoplanktonic bacteria, cyanobacteria and algae are heavily grazed by mixotrophic and heterotrophic organisms (2-200 μm, collectively referred to as microzooplankton), has revealed a planktonic world of intense activity (Stockner and Porter 1988), and challenged our comprehension of the fate of particles and the trace elements that they scavenge in surface waters.

This dissertation focuses attention on the impact of the microbial food web upon trace metal fates in the Laurentian Great Lakes. These great lakes possess 20% of the world's surface fresh water and are thus a significant global resource, yet they are under sustained anthropogenic stresses due to waste disposal and land use practices, to name only a few. To further our understanding of the geochemical cycles of trace metals in aquatic systems, a hypothesis is presented that links current concepts of the biological and geochemical fate of trace metals; this hypothesis is based on the Great Lakes and it is in principle applicable to other similar large water bodies.

1.1 METALS IN LAKES

Trace metal inputs to the water column of lakes can be summarized as atmospheric deposition, riverine inputs, diffusion and seepage from sediments, and sediment resuspension. In large lakes such as the Laurentian Great Lakes, with small watershed to lake surface ratios, atmospheric inputs are the dominant source term for many trace metals (Nriagu 1986, Flegal et al. 1989). Moreover, this input

path becomes more dominant during thermal stratification when surface waters are effectively isolated from the hypolimnion and any sediment-driven influences. For example, an analysis of sediment trap material collected in Lake Michigan pelagic surface waters after the onset of thermal stratification revealed a decreasing abundance of detrital material originating from resuspended sediment or allochthonous sources (Robbins and Eadie 1991).

In the Laurentian Great Lakes, trace metals are present in pelagic surface waters during thermal stratification at concentrations much lower than are predicted on the basis of known solid phase solubilities (Martell and Smith 1993) and metal loading rates (Nriagu et al. 1996). Our current understanding of trace metal fates in these lakes and other large aquatic systems originates from Sillén's (1961) attempt to explain the elemental composition of seawater by invoking the assumption of chemical equilibrium between the dissolved phase and solid phases present in the sediment. This led to the formulation of several "thallasochemical" models based on similar principles (MacIntyre 1970). The principles described by Sillén were applied by Kramer (1964, 1967) to explain the ionic composition of waters in the Laurentian Great Lakes. However, the basis of more recent attempts to model geochemical fates of trace metals is Schindler's original proposition that particles in the water column control the concentrations of these elements (Schindler 1975). Schindler's approach is based on the treatment of particle surfaces as ligands that can react with trace metals in solution. It assumes a condition of steady state where, simply stated, the gravitational losses of metal-laden particles from the water column balance metal inputs from various sources.

The following equations outline the basic principle of trace metal scavenging in aquatic systems:

$$\frac{\delta [M]_T}{\delta t} = \frac{1}{\tau_M} \cdot [M]_T \qquad (1.01)$$

$$\tau_M = \frac{\int C_p \delta z}{F_p} \cdot \frac{[M]_T}{[M]_p} = \frac{\tau_p}{f_p} \qquad (1.02)$$

$$f_p = \frac{K_p C_p}{1 + K_p C_p} \qquad (1.03)$$

where, F_P is the particle flux, $[M]_P$ the particulate metal concentration, C_P the particle concentration, and z the depth. The rate of metal removal from the dissolved phase ($\delta[M]_T/\delta t$) is first order with respect to total dissolved metal concentration in the system, $[M]_T$. The removal rate constant, τ_M^{-1}, is directly proportional to the fraction of metal on (*sic*) particles, f_P, and inversely proportional to the residence time of the particles, τ_P. Thus, given a constant particle residence time in the reference volume of sea water, the rate of metal removal is a function of the intensity of the metal-ion interaction with settling particles; f_P, in turn, is a function of the partition coefficient between solution and particles, K_P (from: Santschi et al. 1993).

This scavenging model specifies a loss of particles from a defined volume of water; the net removal of these particles can be sedimentary loss to the sediments, or in the case of dimictic lakes, to the hypolimnion. Lower limits of τ_M are limited by the degree of the intensity of the metal-ion interaction with settling particles, whereas upper limits are governed by the solubility of trace metal solids and the residence time of the water body.

The master variable in the one-box steady state models based on adsorption-desorption equilibria is the distribution coefficient, K_P (above). Scavenging is the generic term used to describe the net effect of the numerous mechanisms for this partitioning reaction (electrostatic attraction, surface complexation, surface precipitation, and substitution into crystals; Davis and Kent 1990), which to some extent are affected by changes in pH, the type and concentration of ligands in solution and on the particle surface, and temperature. In addition to participating in the aforementioned sorptive mechanisms, living particles can scavenge trace metals through various biological uptake mechanisms, for example, diffusion, facilitated diffusion, and active transport (Simkiss and Taylor 1995). As well, protozoa and mixotrophic algae comprise a significant proportion of the seston in pelagic surface waters; these living particles ingest other particles, inevitably containing trace metals, through endocytosis (*see* Section 1.4).

The principles of surface complexation modeling can be applied to predict the distribution coefficient of a metal between the aqueous phase and a model particle sorbent[5]. However mechanistically valid this approach may be, it has not been adequate for all applications due to its inability to account for changes in the nature of the scavenging phase (particle dynamics), the kinetics of scavenging, and the various mechanisms of trace metal scavenging (the magnitude of the biological uptake of trace metals is not necessarily related to the predicted stability of a surface complex based on the metal's hydrolysis constant (e.g. Ag; Fisher 1986)).

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[5] Santschi, Honeyman, and Quigley (1993) summarize the historical development of trace metal scavenging models originating from Schindler's application of surface complexation modeling to one-box steady state models.

Most notably, the one-box steady state model cannot accommodate a change in the partitioning of a trace metal in the dissolved phase. Within the dissolved phase of natural surface waters can be found an appreciable amount of colloidal material. Through its complexation with trace metals, colloidal matter can alter the availability of these elements for scavenging by surfaces. The model, as outlined above, does not account for the chemical speciation of the trace metal, or for possible changes in the "dissolved" ligand concentration, which is undoubtably subject to its own dynamic cycling processes. These complexation reactions impact significantly on the value for trace metal partitioning into solids, f_P, and an accurate modeling of trace metal fates must account for possible changes in the partitioning of the metal over time (Baskaran et al. 1992).

The central importance of particulate matter flux to the control of trace metal concentrations is well-established. However, the true nature of the scavenging phase is enigmatic. The focus of this dissertation is the nature of the scavenging phase, in particular the microbial component of pelagic Lake Erie surface water during thermal stratification, and how it affects metal partitioning between the particulate and dissolved phases.

1.2 THE IMPORTANCE OF BIOTA AS TRACE METAL SCAVENGERS

Reviews on the distribution of trace metals in oceans (Morel and Hudson 1985, Wangersky 1986, Murray 1987, Whitfield and Turner 1987) argue effectively for the importance of biotic influences on trace metal chemistry in the water column, based on the principle of scavenging by particle surfaces. By extension to large lakes, biota are considered to play a key role in determining the flux of trace metals in the pelagic zone.

A summary of recent studies that have employed trace metal clean protocols for water sampling and analysis reveals very low concentrations of trace metals in the pelagic surface waters of some of the Laurentian Great Lakes during thermal stratification (Table 1.1). In many cases, these metal concentrations are orders of magnitude lower than those measured previously with less rigorous attention to avoiding contamination, and are unexpectedly very low considering: the proximity of the sampling stations to the shoreline (in comparison with the open ocean), the complete vertical mixing of the lakes during isothermal conditions, the relative shallowness of the lakes, and the omnipresent sources of pollution in the Great Lakes watershed. For some elements, the concentrations are below those measured in pelagic oceanic regions (*see* Table 2.1). Evidently, the scavenging of trace metals by plankton in these great lakes can efficiently purge the surface waters of particle reactive trace metals during thermal stratification.

Table 1.1. Summary of reported trace metal concentrations measured in the epilimnion of some Laurentian Great Lakes using trace metal clean protocols for sampling and analysis.

Trace Metal	[M]	Open Water Stations: Superior	Erie	Ontario	City Harbours: Hamilton	Toronto
Cadmium	pM		77 ± 14[a] 13 ± 7[b] 39[d]	18 ± 6[a] 83 ± 132[c] 44 ± 10[b]	20[a]	83[a]
Chromium	nM	1.2 ± 0.3[b] 1.3[e]	2.8 ± 0.3[b] 1.7[d] 2.6[e]	5.1 ± 1.7[c] 7.5 ± 1.4[b] 6.8[e]		
Copper	nM	11.8 ± 0.8[b]	11 ± 2[a] 13 ± 1[b] 7.2[d]	13 ± 1[a] 13[a] 13 ± 3[b] 14 ± 2[b]	16[a]	
Iron	nM	9.9 ± 7.3[b]	8.6 ± 4.3[b] 21[d]	22 ± 17[c] 20 ± 21[b]		
Lead	pM	27 ± 33[b] 123 ± 4[f]	88 ± 12[f] 77 ± 53[g] 28 ± 17[b]	83 ± 25[f] 22 ± 14[g] 55 ± 16[c] 54 ± 27[b]	292 ± 13[g]	97 ± 39[g]
Manganese	nM	2.3 ± 1.7[g]	2.0[d]	3.4 ± 1.3[c]		
Nickel	pM		13 ± 2[b]	15 ± 1[b]		
Thallium	pM	6 ± 1[h]	45 ± 7[h]	28 ± 3[h]	130 ± 21[h]	
Zinc	nM	4.52 ± 3.43[b]	0.63 ± 0.19[a] 0.37 ± 0.05[b] 2.10[d]	0.19 ± 0.07[a] 2.55 ± 0.76[c] 2.90 ± 0.76[b]	0.18[a]	1.75[a]

Note: [a] Coale and Flegal 1989; surface water, 0-1 m. [b] Nriagu et al. 1996; surface water (0-2 m, Lake Erie and Lake Ontario; 2-21 m, Lake Superior). [c] Nriagu et al. 1993; surface water (1-10 m). [d] Stn. 84; 5 m (*see* Section 3.3.3). [e] Beaubien et al. 1994; surface water. [f] Cheam et al. 1992; Lake Superior (12 m), Lake Erie (6 m), Lake Ontario (0-10 m). [g] Flegal et al. 1989; surface water (0-1 m). [h] Cheam et al. 1995; surface water.

Studies on large lakes in Switzerland have shown that trace metals in lake water are scavenged primarily by the organic solid phase (Sigg 1987); bulk chemical measurements of sedimenting particles reveal that this organic matter arises from autochthonous production. In addition, the strong correlation between zinc and reactive silicate profiles in Lake Sammamish (Washington) suggests that the fate of zinc is controlled by diatom biomass (Balistrieri et al. 1992); a similar control mechanism for Zn was observed in lakes Michigan (Shafer and Armstrong 1991) and Windemere (Reynolds and Hamilton-Taylor 1992). In comparison with the open oceans, there has been relatively little systematic study of trace metal distributions in large lakes. A recent survey in the Great Lakes by Nriagu et al. (1996) provides the most detailed picture of the temporal and spatial distribution of dissolved trace elements in lakes Ontario, Erie, and Superior: profiles characteristic of intense scavenging of Zn and Cd were present during periods of high plankton productivity.

1.3 PLANKTON ECOLOGY AND THE MICROBIAL FOOD WEB[6]

The relatively recent (late 1970's) discovery of photosynthetic picoplankton in oceans and lakes around the world has stimulated a significant amount of research into assessing the ecological importance of a group of phytoplankton hitherto overlooked (Stockner and Antia 1986, Stockner 1991). These microalgae and cyanobacteria are highly productive and are grazed primarily by small heterotrophic and mixotrophic organisms. Because of their high production and consumption rates, they are instrumental in the recycling of carbon within the photic zone. This is based on the following scheme (Azam et al. 1983, Ducklow 1983): autotrophic picoplankton fix inorganic carbon and are rapidly consumed by heterotrophic and mixotrophic protozoa with losses of carbon occurring from both predator and prey respiration, and by the excretion of dissolved organic matter (DOM) due to digestive inefficiency. Excreted DOM provides an energy source for heterotrophic bacteria, which like their autotrophic counterparts in the picoplankton, are grazed heavily by microzooplankton. In turn, microzooplankton are grazed heavily by mesozooplankton which allows for further losses of fixed carbon due to digestive inefficiencies and respiration. Therefore, the grazing activity of the microzooplankton does not readily translate picoplankton productivity into biomass at higher trophic levels.

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[6] The microorganisms comprising the planktonic microbial food web are divided into three size-based classifications according to Sieberth et al. (1978). The following definitions are used in the present study: **picoplankton**, organisms 0.2-2 μm; **nanoplankton**, organisms 2-20 μm, and **microplankton**, organisms 20-200 μm; **microzooplankton**, heterotrophic and mixotrophic organisms 2-210 μm; and **mesozooplankton**, heterotrophic organisms >210 μm.

A recent report on the ecology of the picocyanobacterium *Synechococcus* in the Laurentian Great Lakes (Fahnenstiel et al. 1991a) provides insight into the possible role of picoplankton in determining trace metal fates in lakes. This picoplankter has an *in situ* specific growth rate of 0.1 - $0.9 \cdot d^{-1}$ and accounts for 1-26% of the primary production in lakes Huron and Michigan. In comparison, Fahnenstiel et al. (1986) attribute 50% of the primary production in Lake Superior to the plankton fraction <3 μm. Grazing losses have been measured to be 33-120% of the growth rate of *Synechococcus*; 68% of this loss is due to small (4-10 μm) heterotrophic nanoflagellates (e.g. *Ochromonas*) and ciliates, whereas only 5-21% of the losses to grazing are accounted for by crustaceans and rotifers (Fahnenstiel et al. 1991a). Members of the microbial food web are also present in lakes Erie and Ontario (Caron et al. 1985, Pick and Caron 1987, Weisse and Munawar 1989). The importance of the microbial food web is inversely proportional to the degree of nutrient enrichment (Pick 1991, Weisse 1991). Hence, with the onset of thermal stratification, following the loss of nutrients from the epilimnion by the sedimentation of diatom biomass after the spring bloom, the prevailing conditions favour an active microbial food web.

1.4 TRACE METAL FATE: LINKING ECOLOGICAL AND GEOCHEMICAL FATES

The microbial food web must have a major influence on the fate of trace metals in lake water. Due to the rapid growth rate of autotrophic and heterotrophic picoplankton, the high surface area to volume ratios of these small organisms, and the high affinity of trace metals for biological surfaces (Fisher 1986, González-Dávila 1995), picoplankton have an extremely high potential to scavenge trace metals from the dissolved phase (Fisher 1985). Consumption of picoplankton by protozoa is by phagocytosis, whereby the prey are engulfed *in toto*.[7] In accordance with the ecological fate of

[7] There exist numerous ways that protozoa capture and consume their prey. These are centred on three general themes (Fenchel 1987): "filter feeding", the use of cilia or pseudopodia for straining water for particles; "direct interception", raptorial-type interception of prey in the surrounding water, often with the use of a flagellum to entrap the prey and bring it to the cell surface for phagocytosis (e.g. the chrysophyte *Epipyxis pulchra*; Wetherbee and Andersen 1992); and "diffusion feeding", the dependence upon food items to randomly encounter the predator. Consumption is often by phagocytosis although other related methods are used, especially for thecate dinoflagellates, e.g. the extrusion of a feeding veil (pallium) to enrobe prey in an amoeboid-type manner for extracellular digestion (e.g. the heterotrophic dinoflagellate *Protoperidinium spinulosum*; Jacobson and Anderson 1992), and the use of the peduncle (a protoplasmic extension) by dinoflagellates for penetrating the cell wall of a prey (the peduncle serves as a conduit for the transport of the cytoplasmic contents of the prey into the predator's cell).

picoplankton dominated by consumption by microzooplankton, the majority of metal scavenged by picoplankton must enter a larger particle size fraction.[8]

The digestive cycle among protozoa is similar (Capriulo 1990). Prey items consumed by phagocytosis are retained in an endocytotic vacuole formed by the invagination of the cell membrane. The vacuole either becomes a digestive vacuole or fuses with an existing one. Digestion proceeds under acidic (pH ≈2) conditions. Material within the digestive vacuole is transported to other parts of the cell by vesicles budding off from the digestive vacuole, and further digestion is marked by an increase in alkalinization of the vacuole. However, the assimilation of ingested material is not complete (Caron and Goldman 1990). Colloidal and particulate wastes are excreted from protozoa by ejection vacuoles (Capriulo 1990), the loss of digestive vacuoles upon cell division (*see* Chapter Two), and perhaps through the activity of contractile vacuoles. Since this excretion serves to regenerate elements for re-assimilation by prey, protozoan grazing activity is a process for the recycling of elements.

Trace metal regeneration by consumer organisms in the microbial food web is obligatory. Macronutrients and micronutrients are present in planktonic biomass in a ratio which reflect the balance between nutritional needs, nutrient storage, and the limits of toxicity for some trace elements which are unavoidably assimilated by cells (Morel and Hudson 1985). This stoichiometric ratio was first revealed for the major nutrients (C_{106}:N_{16}:P) by Redfield (1934), and it has since been refined and extended to include several trace metals (Table 1.2). If we assume similar cellular elemental composition for protozoa and phytoplankton, then one can readily demonstrate that regeneration of micronutrients must occur in order for the consumer organism to maintain Redfield proportions of trace metal in its biomass. Let us consider the case of a generic heterotrophic nanoflagellate grazing a generic cyanobacterial picoplankter, and the fate of the essential, yet potentially toxic element, copper. If a gross growth efficiency of 30% is assumed, then the required regeneration of copper must be at least 70% of the consumed copper obtained from the prey biomass, in order to avoid exceeding the normal homeostatic concentration of cellular copper as the nanoflagellate organism seeks to double its carbon quota for cellular division. This is a lower limit for regeneration since this calculation involves the unlikely assumption that the unicellular nanoflagellate accumulates no copper from the dissolved phase.

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[8] Although grazing is generally limited by size (e.g. picoplankton are primarily grazed by consumers in the nanoplankton) there are exceptions to these rules. Smaller organisms have been known to consume larger ones, and the consumption of prey by grazers in the same plankton size class is possible.

Table 1.2. Reported Redfield ratios for plankton biomass extended to include trace metals.

Ratio	Source
$C_{106}:N_{16}:P:Zn_{0.03}:Cu_{0.006}$	–field samples of collected settling material in Lake Zurich (Sigg 1987)
$C_{106}:P:Zn_{0.04\text{-}0.1}:Cu_{0.002\text{-}0.003}$	–field samples of collected settling material in Lake Windemere (Reynolds and Hamilton-Taylor 1992)
$C_{106}:N_{16}:P:(Fe, Mn, Zn)_{0.01}:(Cd,Cu,Ni)_{0.001}$	–laboratory studies of trace metal toxicity/limitation thresholds for individual neritic marine phytoplankton (Morel and Hudson 1985)
$C_{106}:N_{16}:P:Fe_{0.005}:Zn_{0.002}:(Cd,Cu,Mn,Ni)_{0.0004}$	–field samples collected from pelagic marine environments (Bruland et al. 1991)

Microzooplankton grazing regenerates appreciable amounts of macronutrients (e.g. N and P; Caron and Goldman 1990). Like the argument for the obligatory regeneration of trace elements, the assimilation efficiency for organic carbon leads to the consumption of macronutrients at levels that exceed physiological needs. Therefore, since protozoan grazing regenerates macronutrients, it follows that protozoan grazing must regenerate trace metals from their prey as well. However apparently evident this may seem, it remains to be established empirically. In addition, the relative degrees of regeneration among various physiologically essential and non-essential trace metals cannot be derived from this argument alone.

If trace metals are regenerated then this will have an impact on their geochemical fate. Since the exocytosis of waste from digestive vacuoles in protozoa releases appreciable amounts of organic colloidal and particulate matter (Stoecker 1984, Tranvik 1994) any trace metals excreted in such a fashion are likely to be bound by these compounds. Trace metal complexation by waste generated by microzooplankton grazing is expected to increase the residence time of these elements in two ways. Firstly, an increase in the dissolved pool, $[M]_T$, of which the colloidal fraction is a significant fraction for some trace metals (Morel and Hering 1993, p.364; Sholkovitz 1995), will increase τ_M if the particle flux remains the same. This assumes that trace metals complexed to organic colloids have a lower affinity for particle surfaces than the free ion. Secondly, the recycling of excreted trace metal back into the pool of metal available for scavenging reactions would increase the average time required for a given atom of metal to leave the surface water. Therefore, if microzooplankton grazing is active in surface waters then one would expect that the geochemical fate of those trace metals that are associated with the prey, and consumed during this trophic interaction, will inevitably be affected. Hence, the

ecological fates of plankton in the microbial food web and the geochemical fates of trace metals scavenged by this plankton must be closely linked.

1.5 FORMULATION OF THE HYPOTHESIS

The preceding arguments are summarized as follows:

- Because of their size, abundance, and rapid growth rates, picoplankton should play an important role in the scavenging of trace metals from the dissolved phase in surface waters.
- Picoplankton are grazed intensely by consumers in the microbial food web; this grazing has been shown to regenerate macronutrients such as P and N.
- Stoichiometric considerations based on Redfield proportions of trace elements and carbon in plankton biomass, and physiological considerations of protozoan carbon-based growth efficiencies which are less than unity, support the argument that the grazing of prey by microzooplankton will lead to the regeneration of the trace metals consumed with the prey.
- Regenerated particle-reactive metals are likely to be less available for scavenging reactions than the form of metal initially scavenged by the plankton, and are thus less likely to rapidly leave the system. Therefore, trace metal regeneration by microzooplankton grazing is expected to increase the residence times of these elements in surface waters.

These arguments lead to the following hypothesis regarding the role of the microbial food web in determining the fate of trace metals in surface waters:

HYPOTHESIS

> Microzooplankton can regenerate significant amounts of trace metal into the dissolved phase through the incomplete assimilation of trace metals from their prey during digestion. It follows that the grazing activity by this collective group of organisms will increase the residence time of those trace metals in surface waters that are scavenged by the prey of microzooplankton.[9]

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[9] When this hypothesis was formulated in late 1991, the idea was novel–no studies, in freshwater science or marine science, had directly addressed this area of study. Since that time several reports which conclude that trace metal regeneration by protozoa occurs in marine systems have been published (*see* Chapter 6 *for a summary of these studies*). Whereas earlier reports lacked a definitive demonstration of trace metal regeneration by protozoa, the reports based on this dissertation provide the first unequivocal demonstration of trace metal regeneration by protozoa, both under controlled laboratory conditions (Twiss and Campbell 1995) and in natural waters (Twiss et al. 1996).

1.6 OBJECTIVES

I. The primary objective of this dissertation was to establish that microbial food web organisms can regenerate a suite of trace metals into the dissolved phase during their digestion of prey items which contain these elements. To this end, a controlled laboratory experiment was designed using a simplified model microbial food web composed of two organisms (a predator and a prey) of ecological significance in the Laurentian Great Lakes.

The trace metals chosen for study were cesium, cadmium, zinc, and gadolinium. These metals represent a range of charge to ionic radius ratios (z^2/r: Cs = 0.6, Cd = 4.1, Zn = 5.4, Gd = 9.6) and accordingly, a range of reactivities with surfaces (Gd > Zn, Cd >> Cs). In addition, these elements represent a range in nutritive worth: from the essential micronutrient Zn to the relatively toxic Cd, and the absence of any reported nutritive requirements or toxicity for Cs and Gd.

II. The second objective was to verify if microzooplankton grazing on trace metal partitioning has a measurable effect in the natural environment. Thus, an extension of the experimental design used in the laboratory was carried out in pelagic Lake Erie.

III. Finally, to evaluate and predict the impact that microozooplankton can have on trace metal geochemistry in large lakes, a model of trace metal fates in relation to the microbial food web was developed based on observed plankton and trace metal behaviours in pelagic Lake Erie. The modeled outcome of trace metal residence times was compared to residence times estimated in the absence of microbial food web activity.

1.7 LIMITATIONS TO THE SCOPE OF THE INVESTIGATION

This dissertation research is limited to the pelagic surface waters of large lakes during thermal stratification. In large lakes, such as the Laurentian Great Lakes, influences from sediment-driven processes, which strongly affect trace metal distributions in small lakes, are reduced during the stratification period. Hence, water chemistry in large lakes is usually more stable than in smaller water bodies and autochthonous particles will be the dominant scavenging phase. The present evaluation of the importance of the microbial food web, with regards to trace metal fates, is therefore applicable only to water bodies with similar physical and biological characteristics, i.e. surface water physically

removed from the sediment or underlying water layers by stratification, and particle composition dominated by biota.

Only microbial food web organisms (<210 μm) are studied here as players in regenerative processes. This is not to disregard the potential influences of viral lysis of plankton, regeneration by mesozooplankton grazing, or the impact that these factors may have on the dynamics of the microbial food web. Instead, to achieve a primary understanding of the impact that the microbial food web has on trace metal dynamics, the focus here is only on the activity within this food web.

The metals chosen for study (Cs(I), Cd(II), Zn(II), Gd(III)) are insensitive to changes in oxidation-reduction potential under the conditions of this study. Therefore, any change in bioavailability is assumed to be due to the effect of the metal cation binding to ligands and not to a change in the redox state of the metal which might alter its bioavailability, such as the case under surface water conditions for some other trace metals, e.g. Ce, Co, Cu, Fe, and Mn.

1.8 OUTLINE OF THESIS

In Chapter Two the premise that protozoan grazing leads to the regeneration of trace metals contained within the prey is validated through the demonstration of the regeneration of several trace metals (Cs, Cd, Zn, Gd) in a simplified microbial food web. The two microorganisms chosen for study represent two dominant genera of the Laurentian Great Lakes microbial food web, the mixotrophic nanoflagellate *Ochromonas danica* (a predator) and the picocyanobacterium *Synechococcus leopoliensis* (a prey).

In Chapter Three these observations are confirmed in the field (pelagic surface waters of Lake Erie). The grazing of ^{109}Cd- and ^{65}Zn-radiolabeled *Synechococcus leopoliensis*, introduced into sampled lake water to trace the ecological fate of the natural picoplanktonic community, leads to the regeneration of the radionuclides into the dissolved phase, and the trophic transfer of these elements into the nanoplankton and microplankton.

Chapter Four describes an investigation into the scavenging of Cs, Cd, Zn and Gd by seston in the surface waters of pelagic Lake Erie during thermal stratification. The partitioning of these trace metals between the seston and dissolved phase is followed by measuring the metal scavenged by each of the ecologically significant plankton size classes: the picoplankton, the nanoplankton, and the microplankton.

Chapter Five describes the development of static and dynamic plankton models based on empirical data and the principles of a steady state. These models link the ecological fate of plankton in the microbial food web with the geochemical fates of trace metals. The dynamic plankton model is used to evaluate the effect of the microbial food web on the residence times of Cs, Cd, Zn and Gd in the surface waters of Lake Erie, and to predict how changes to plankton community structure could influence trace metal residence times.

In Chapter Six, the data in support of the hypothesis are summarized and the hypothesis is critically evaluated for its relevance, testability, applicability, accordance with other well-established hypotheses, and predictive worth.

CHAPTER TWO

REGENERATION OF TRACE METALS FROM PICOPLANKTON BY NANOFLAGELLATE GRAZING: A LABORATORY STUDY BASED ON A SIMPLIFIED LAURENTIAN GREAT LAKES MICROBIAL FOOD WEB[1]

2.1 ABSTRACT

Rapid regeneration of trace metals from the particulate to the dissolved phase (<0.2 μm) was observed in the laboratory with a simplified microbial food web composed of mixotrophic chrysophycean nanoflagellates (*Ochromonas danica*) grazing on picocyanobacteria (*Synechococcus leopoliensis*) that had been previously exposed to the radionuclides ^{153}Gd(III), ^{65}Zn(II), ^{109}Cd(II) and ^{137}Cs(I). These trace metals were chosen to represent a range of surface reactivities with particles (Gd > Zn, Cd >> Cs). Grazing experiments and the appropriate non-grazing controls were carried out in batch cultures over 43-49 hours in defined, inorganic freshwater medium; metal partitioning among the consumer, prey and dissolved phases was determined by sequential filtration (3 μm, 0.2 μm) at timed intervals. The majority of trace metals consumed as radioactive prey were regenerated into the dissolved phase. Regenerated Gd, Zn and Cd present in the dissolved phase were less available for resorption by plankton than were the same radionuclides added in inorganic form to fresh growth medium. These results suggest that, where this grazing activity exists, it will serve to increase trace metal residence times in the water column.

2.2 INTRODUCTION

Our appreciation of the biogeochemical cycling of trace metals in natural waters has changed radically in recent years, with respect both to the concentrations involved and to the mechanisms implicated in controlling these concentrations. For example, using trace metal clean protocols developed for determining metal levels in the open ocean, researchers have recently demonstrated that levels of dissolved Cd, Cu, Pb and Zn in the surface waters of the lower Laurentian Great Lakes of North America are very low (Coale and Flegal 1989, Nriagu et al. 1993), several orders of magnitude less than previously reported. Since these low concentrations generally lie far below the levels that

[1] Twiss, M.R., and Campbell, P.G.C. 1995. Regeneration of trace metals from picoplankton by nanoflagellate grazing. Limnol. Oceanogr. 40: 1418-1429.

would be predicted from the precipitation-dissolution equilibria of known solid phases, surface complexation of metals by ligands on particle surfaces has been proposed as an alternative mechanism controlling trace metal concentrations in natural waters (*see review in* Santschi et al. 1993). The gravitational flux of these metal-laden particles out of the water column would then maintain the low concentrations of trace metals observed in the pelagic regions of large lakes and oceans (Table 2.1).

Table 2.1. Dissolved metal concentrations found in the pelagic regions of the lower Laurentian Great Lakes and the northern oceans.

	Gd (pM)	Zn (nM)	Cd (pM)	Cs (nM)
Lake Erie	---	0.39 - 0.84[a]	72.4 - 96.6[a]	---
Lake Ontario	---	0.07 - 0.62[a]	6.3 - 31.9[a]	---
		2.1 - 3.9[b]	44 - 273[b]	---
North Atlantic	5.6[c]	0.06[d]	2[d]	2.2[e]
	4.9[f]			
North Pacific	4.0[g]	0.23[h]	2.8[h]	---

Note: [a] surface; Coale and Flegal 1989. [b] 1-25 m; Nriagu et al. 1993. [c] surface; Elderfield and Greaves 1982. [d] surface; Bruland and Franks 1983. [e] surface; Bruland 1983. [f] 10 m; de Baar et al. 1985a. [g] 15 m; de Baar et al. 1985b. [h] surface; Bruland et al. 1994.

In large lakes, such as the Laurentian Great Lakes, influences from sediment-driven processes, which strongly affect trace metal distributions in small lakes, are reduced. Hence, water chemistry in large lakes is usually more stable than in smaller water bodies. The distribution of trace metals in oceans is strongly affected by biotic influences (Morel and Hudson 1985, Whitfield and Turner 1987); similar mechanisms might be expected to dominate in the pelagic regions of large lakes. Indeed, recent studies on large lakes suggest that autochthonous particles do play a key role in the adsorptive control of dissolved metals (Sigg 1987). Depending on their size and composition, these particles may either be recycled in the epilimnion or lost to the bottom sediments.

The microbial food web is an important trophic link in both marine and freshwater systems (Stockner 1988). The consumers in this food web are the microzooplankton, e.g. rotifers, dinoflagellates, ciliates, heterotrophic and mixotrophic nanoflagellates, less than 200 μm in size. This group of consumers has been shown to regenerate macronutrients (notably phosphorus and nitrogen) in the marine environment (*cf.* Caron and Goldman 1990). Similarly, microzooplankton grazing can regenerate P in freshwater environments (Taylor and Lean 1981, Rothhaupt 1992). The picoplanktonic

organisms (algae, cyanobacteria, and bacteria; 0.2-2 μm) preyed on by microzooplankton have an enormous potential to scavenge and assimilate dissolved trace metals, owing to their high surface area to volume ratios (Fisher 1985) and rapid growth rates. Although there is preliminary evidence for regeneration of Fe by marine microzooplankton (Hutchins et al. 1993, Hutchins and Bruland 1994), to date no studies have directly shown that microzooplankton can regenerate into the dissolved phase the other trace metals that they ingest as an integral fraction of their prey.

To illustrate the influence that microbial organisms could have on trace metal geochemical cycles in large lakes, a microbial food web was manipulated under controlled conditions to demonstrate that microzooplankton can regenerate significant fractions of trace metal into the dissolved phase through their grazing activity. This simplified food web was composed of two organisms, each representative of key primary producers and consumers in the Laurentian Great Lakes (Fahnenstiel et al. 1991a). The experimental system consisted of the nanoflagellate *Ochromonas danica* grazing on the picoplankter *Synechococcus leopoliensis* which had been previously labeled with the gamma-emitting radionuclides ^{137}Cs(I), ^{109}Cd(II), ^{65}Zn(II), and ^{153}Gd(III), a rare earth element. Elemental fractionation of the trace metals by grazing activity was then determined by differential size filtration. The trace metals used here represent a range of charge to ionic radius ratios (z^2/r: Cs = 0.6, Cd = 4.1, Zn = 5.4, Gd = 9.6) and accordingly, a range of reactivities with surfaces (Gd > Zn, Cd >> Cs). According to the depth profiles of these elements in the open ocean, cesium is classified as conservative, or accumulated in the water column, whereas the other elements are recycled, i.e. scavenged by sinking particles at the surface but remineralized at depth (Whitfield and Turner 1987); similar behaviour of these elements, with the exception of Cs, is expected in freshwater.

2.3 METHODS

2.3.1 Culture pretreatment

The picocyanobacterium *Synechococcus leopoliensis* (UTEX 625, referred to herein as *Synechococcus*) growing exponentially ($\mu \approx 1 \cdot d^{-1}$) in Fraquil medium (Morel et al. 1975) was harvested onto 0.4 μm polycarbonate membrane filters (Nuclepore) and rinsed with FRAt (Fraquil medium with no FeEDTA or trace metals added). The applied vacuum was <13 kPa to minimize cell breakage. Cells were resuspended ($\approx 5 \times 10^6$ cells$\cdot$mL^{-1}) in 500 mL rFRAt (radioactively labeled FRAt) in 1 L acid-washed polycarbonate flasks. The medium had been spiked 24 h previously with 40 microliters of a radioactive cocktail containing 37 kBq ^{137}Cs, 25 kBq ^{153}Gd, 29 kBq ^{109}Cd, and 23 kBq ^{65}Zn in 0.5 M

HCl. Forty microliters of 0.5 M NaOH was added immediately before the radioactive spike to neutralize the addition of acid. Nominal metal concentrations after addition were 1.2 nM Gd, 16.3 nM Zn, 11.2 nM Cd, and 0.54 nM Cs.

Cultures were maintained at 20 °C with continuous illumination of 95 μmol photons·m^{-2}·s^{-1}, and gyratory shaking (50 rpm). After 2-3 d, the entire flask contents were harvested by filtration (as above) and transferred to 10^{-4} M Na_2EDTA (pH 7.6) for 15 min in order to remove any weakly adsorbed radionuclides. Cells were filtered once more, rinsed with FRAt, and resuspended in FRAt.

The chrysophyte *Ochromonas danica* (UTEX 1298, referred to herein as *Ochromonas*), growing exponentially ($\mu \approx 1.5 \cdot d^{-1}$) in Ochromonas medium (Starr and Zeikus 1993) under conditions identical to those for *Synechococcus* (except no shaking), was harvested by centrifuging for 10 min at 3,000 rpm using a table top centrifuge. The supernatant was discarded and the pellet was rinsed and transferred to 10^{-4} M Na_2EDTA (pH 7.6). After 5 min the cells were centrifuged, the EDTA supernatant decanted, and the pellet was rinsed and resuspended in FRAt; this wash was used to remove any residual trace metals on cell surfaces. Microscopical observation at this point showed the cells to be spherical and to contain large vacuoles. Flagella were attached to many cells and little cell debris was present.

2.3.2 Metal partitioning in *Synechococcus*

The internalization of radionuclides by the prey cells during the pretreatment was assessed by exposing *Synechococcus* to radionuclides and measuring accumulated trace metals over time. *Synechococcus* cells growing exponentially in Fraquil were rinsed with FRAt and suspended in rFRAt (prepared as above) to give a density of 3.4 x 10^5 cells·mL^{-1}. At timed intervals over 24 h, the culture was sampled for cell density and total radioactivity, and triplicate 20-mL samples were filtered onto 0.2 μm filters (Nuclepore) and rinsed with 10 mL FRAt. In addition, a second set of triplicate 20-mL cell samples were exposed to 10^{-4} M Na_2EDTA solution for 20 min and then filtered as above. Correction for passive retention of radionuclides by the filter membrane was achieved by using superimposed filters, with the bottom filter serving as the control for radionuclide sorption by the filter membrane. The use of a 20-min EDTA cell wash and superimposed filters is similar to the approach employed by Schenck et al. (1988), who found that this technique gave similar values for adsorbed metal in comparison with an isotope exchange method. This approach yields values directly for total accumulated radionuclides (C_T) and for the cellular fraction (C_C; operationally defined as non EDTA

extractable). The adsorbed fraction (C_A) was determined by difference (C_T - C_C); the standard deviations of the estimated C_A values were propagated from the standard deviations of the C_T and C_C values.

2.3.3 Grazing experiment

Three treatment flasks were prepared consisting of *Synechococcus* alone (prey control), *Synechococcus* and *Ochromonas* (grazing treatment) and *Ochromonas* alone (predator control). One-liter polycarbonate flasks containing 1 liter of sterile FRAt were inoculated with cell suspensions of radioactive *Synechococcus* and/or unlabeled *Ochromonas*, as prepared above. Initial cell concentrations were $\approx 2 \times 10^6$ *Synechococcus*·mL^{-1} and $\approx 10^4$ *Ochromonas*·mL^{-1}. Treatment cultures were maintained under the same growth conditions outlined above but the flasks were not subjected to gyratory shaking in order to avoid clumping of the cells.

At intervals (t = 0, 4, 9, 23, and 43 h), flask contents were gently mixed and sampled for determination of cell density and sequential size fractionation of radionuclide activity. (Sequential filtration was shown to give identical results to parallel filtration. However, the error in estimating the content of the 3-0.2 μm size fraction using parallel filtration was noticeably greater.) Triplicate samples of radioactivity in the >3 μm, 3-0.2 μm, and <0.2 μm size fractions, in addition to total aqueous radioactivity, were collected from each radioactive treatment flask. Radionuclide partitioning was determined by filtering 50 mL of culture onto a single 3-μm polycarbonate membrane filter (47 mm, Nuclepore). The 3-μm pore size was chosen to optimize the simultaneous retention of *Ochromonas* and passage of *Synechococcus*; in preliminary experiments retention of cells on 3-μm filters was determined to be 91 ± 5% for *Ochromonas* and 1.5 ± 0.2% for *Synechococcus* (mean ± SD). Filtrate (<3 μm) was removed and the still moist 3-μm filter was then rinsed with 5 mL of FRAt and, before it was removed, a very slight vacuum (<5 kPa) was briefly applied to break the surface tension of water between the filter support and the membrane filter. The rinse was not collected but directed into a waste stream. Twenty milliliters of the <3-μm filtrate was passed (<13 kPa) through a 0.2-μm polycarbonate membrane filter (47 mm, Nuclepore) and the filtrate (<0.2 μm) was removed and sampled for radioactivity. The 0.2-μm filter was then rinsed with 5 mL of FRAt and removed. The entire experiment was repeated three times (Expts. 1, 2, and 3) on separate dates over a period of 3 months.

Filtrate (<0.2 μm) from the first and final sampling intervals was further size fractionated with 5 kD molecular-weight cutoff ultrafiltration membranes (ultrafiltration centrifuge filters, Ultraspin 8000, Lida Corp.). Prior to ultrafiltering the filtrate, 100 μL of a non-radioactive solution (pH 7.1) containing 56 μM Cs, 33 μM Cd, 34 μM Zn, and 24 μM Gd was passed through the ultrafiltration device. This pretreatment was designed to minimize losses of radionuclides by adsorption on the cellulose triacetate membrane filter (in preliminary trials 98, 97, 93, and 72% of inorganic radiolabeled Cs, Cd, Zn, and Gd passed the 5 kD-ultrafilter, respectively). Excess pretreatment solution was removed and the filters were tared. Radioactive ultrafiltrate (400 μL, <0.2 μm) from the experimental treatments was then ultrafiltered. The ultrafiltrate (<5 kD) collected was transferred to a sample vial and the ultrafiltrate collection tube rinsed with 5% HCl to ensure a high degree of ultrafiltrate recovery. Radioactivity in the filtrate (<5 kD) was normalized to the volume filtered (400 microliters) minus the volume retained in/on the filter matrix (as determined by the mass of filtrate retained on the tared filter).

2.3.4 Bioavailability to *Synechococcus* of trace metals in filtrate from grazing experiments

To further characterize the filtrate (<0.2 μm) from the grazing treatment, a short-term bioaccumulation experiment was performed on this filtrate. Filtrate (<0.2 μm) collected after 49 h from the *Synechococcus* and *Ochromonas* grazing treatment from Expt. 3 was measured for radioactivity. The radioactivity of each radionuclide in the filtrate was matched by spiking fresh FRAt medium contained in a separate flask with appropriate amounts of individual radionuclide solutions. After 16 h equilibration at 4°C, the filtrate and rFRAt were brought to room temperature and the two flasks inoculated with *Synechococcus* to give an initial density of 10^6 cells·mL^{-1}. After a 5 h exposure time, aliquots from each treatment were filtered onto superimposed 0.2-μm polycarbonate membrane filters and rinsed with 10 mL of FRAt. The 5-h exposure period was chosen because measurable accumulation of metals occurs within this time period (*cf.* Fig. 2.1) and also to minimize growth-induced differences in particle densities between treatments (fresh FRAt medium had a greater concentration of macronutrients than did the filtrate from the grazing treatment).

2.3.5 Trace metal regeneration efficiency of nanoflagellate grazing

The ability of *Ochromonas* to regenerate trace metals consumed with their prey was assessed by relating radionuclides entering the dissolved phase in proportion to the amount consumed by the

grazer over a short term, i.e. 9-16 h following the initiation of the treatments. Regeneration efficiency was calculated as the regeneration of trace metals into the dissolved phase (<0.2 μm), divided by the total quantity of metal consumed with the prey (the difference in *Synechococcus* cell density in grazing and control treatments multiplied by the measured average radionuclide quota of *Synechococcus* during this time period); i.e. (dissolved/consumed) x 100. Dissolved concentrations in the grazing treatments were corrected for non-grazing related radionuclide desorption, as measured in the prey-control treatments. Grazing rates were calculated as the difference in the net population specific growth rates ($\mu = \delta ln$ cell·mL^{-1}/δt) of *Synechococcus* in the prey control and grazing treatments.

2.3.6 Radioactivity measurements

Radioactivity of filters and aqueous samples was determined with a gamma-spectrometer (LKB Wallac Compugamma 1282; NaI crystal) equipped with a multi-isotope assay option (UltroTerm software). Aqueous samples (filtrates and totals) were 2-mL volumes; in the case of the ultrafiltrate (400 microliters) the volume was adjusted to 2 mL to ensure a comparable geometric counting environment with other aqueous samples. The radioactivity of individual radionuclides was measured in the following energy ranges: ^{109}Cd, 20-38 keV; ^{153}Gd, 125-189 keV; ^{137}Cs, 628-756 keV; and ^{65}Zn, 990-1268 keV. Background radioactivity (medium or filter blanks) was subtracted from the sample counts. Propagated errors determined from the error of the sample and background counts were less than 10% (P <0.05) unless noted.

2.3.7 Cell enumeration

Synechococcus cell volume and number was determined using an electronic particle counter (Coulter Multisizer II, 15 μm orifice). Cell density of *Ochromonas* was determined with a haemocytometer on undiluted samples. All counting was done on samples that had been fixed with Lugol's iodine (1.5%).

2.3.8 Glassware preparation

All plasticware and silanized glassware were soaked in 15% HNO_3 and rinsed with deionized water. High-purity deionized water (>17.5 Mohms·cm^{-1}) used in preparing stock solutions and media was obtained from a commercial system employing mixed-bed ion exchange, charcoal adsorption, and

filtration (0.2 μm) steps. Defined media were prepared with reagents of analytical grade or better. Whenever feasible, manipulations were conducted in a filtered air (HEPA) laminar flow cabinet.

2.4 RESULTS

2.4.1 Metal uptake by *Synechococcus*

Measurement of radionuclide accumulation over time, during the pre-labelling phase of the experiment, showed that internal cell quotas reached maximum values within <24 h (Fig. 2.1); Gd exhibited more rapid kinetics, achieving a plateau within about 8 h. Cellular accumulations of metal decreased in the sequence Gd > Cd > Zn >> Cs, as measured by volume concentration factors (VCF; $VCF = [M]_{algae}/[M]_{water}$; Table 2.2).

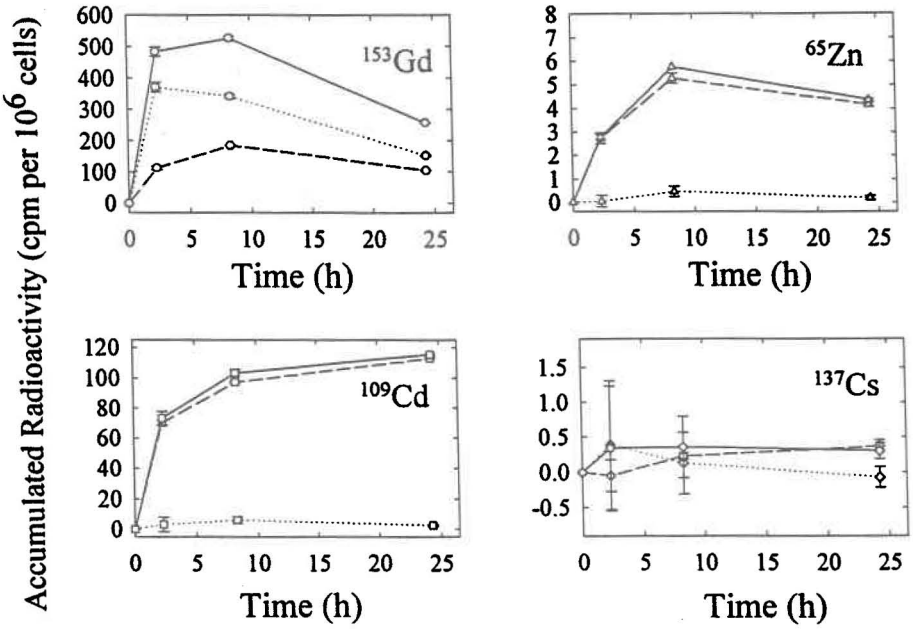

Figure 2.1. Accumulation and partitioning of radioactively labeled trace metals by *Synechococcus* in defined inorganic medium, pH 7.5. Metal concentrations after additions were 1.2 nM Gd, 16.3 nM Zn, 11.2 nM Cd and 0.54 nM Cs. Values are mean ± SD, $n = 3$; error bars are shown only when larger than the size of the symbol. Total cell metal = solid line; adsorbed (EDTA extractable) metal = short dash; cellular metal (total - adsorbed) = long dash.

Adsorption of metals to the cell surface of *Synechococcus* was positively correlated with surface reactivity (Table 2.2), as inferred from the first hydrolysis constants for each metal. After the 24-h incubation period, metal partitioning between the exterior and interior of the algal cells differed among the metals studied. Gadolinium was primarily adsorbed to the cell surface whereas the majority

of Cd and Zn was internalized (i.e. not extracted by EDTA). Cesium partitioning showed no significant difference between total and cellular Cs at an exposure level of 0.54 nM (Fig. 2.1), but since EDTA has little affinity for Cs^+ the EDTA rinse step was probably not more effective than a water-only rinse.

Table 2.2. Volume concentration factors (VCF) for *Synechococcus* exposed to 0.54 nM Cs, 0.82 nM Gd, 9.0 nM Cd, and 16.4 nM Zn in rFRAt medium, pH 7.5 for 24 h. Metal adsorbed to cell surfaces was determined using a 10^{-4} M EDTA rinse. nsd: No significant difference between cellular and total Cs quotas. The amount of adsorbed Cs is thought to be low, but it could not be determined since EDTA is ineffective in complexing Cs^+; for Cs, the EDTA rinse was equivalent to a water-only rinse.

	Gd(III)	Zn(II)	Cd(II)	Cs(I)
$\log_{10}$ VCF	6.4	5.1	5.5	2.9
Adsorbed to cell surface (24 h), %	59 ± 0.5	4.4 ± 2.2	2.2 ± 0.5	nsd
log $K_{M\text{-}OH}$[a]	6.3	4.7	3.9	no ev cpx[b]

Notes: [a] log $K_{M\text{-}OH}$ = log stability constant of the first hydrolysis product (Martell and Smith 1993) expressed at infinite dilution ($K_{M\text{-}OH} = [M\text{-}OH]/[M^{z+}]\cdot[OH]$). [b] No evidence for the formation of a CsOH complex.

2.4.2 Metal loadings

The trace metals added to the grazing and control treatments were in particulate form as radiolabeled *Synechococcus* cells. Expressed as total metal concentrations, metal loadings in these treatments were: 22 pM Gd, 2.4 nM Zn, 0.69 nM Cd, and 0.53 pM Cs (e.g. Expt. 1).

2.4.3 Growth of predators and prey alone and together

The picocyanobacterium *Synechococcus* grew alone at a slower specific growth rate in the control treatment ($0.73\cdot d^{-1}$; Fig. 2.2) than expected ($\approx 1\cdot d^{-1}$ in the stock culture). This decrease may be attributed to a lack of micronutrients in the FRAt medium and possibly to growth inhibition due to the pre-treatment with Cd, Zn and perhaps Gd.

In the presence of *Ochromonas*, the *Synechococcus* population had a net negative specific growth rate of $1.73\cdot d^{-1}$; Fig. 2.2), due to the grazing pressure of *Ochromonas*. Consistent with this interpretation and with microscopical observations of micrograzing activity, *Ochromonas* had a greater net specific growth rate in the presence of *Synechococcus* ($0.86\cdot d^{-1}$; Fig. 2.2) than in its absence

($0.55 \cdot d^{-1}$; Fig. 2.2). It is concluded that the increased specific growth rate and higher cell numbers (5.25×10^4 vs. 1.95×10^4 cells$\cdot$mL^{-1}) of the *Ochromonas* population after 43 h in the presence of *Synechococcus* were due to its grazing of the picocyanobacteria. Moreover, cells of *Ochromonas* grazing *Synechococcus* were ovoid and much larger than the fusiform and elongated *Ochromonas* cells observed in the absence of prey.

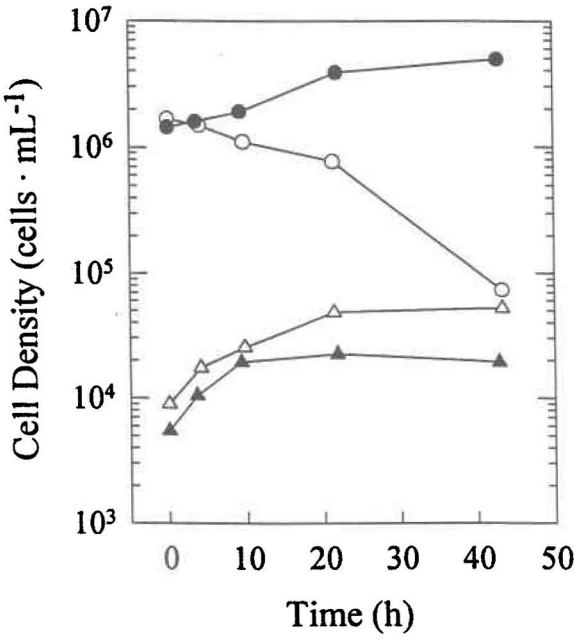

Figure 2.2. Net population growth of *Synechococcus* and *Ochromonas* in experimental treatments from Experiment 1. Symbols: ●–*Synechococcus* alone; ○–*Synechococcus* with *Ochromonas*; ▲–*Ochromonas* alone; Δ –*Ochromonas* with *Synechococcus*.

2.4.4 Metal partitioning between the particulate and dissolved phases: Prey-control treatment

In the absence of grazing pressure, the partitioning of ^{109}Cd and ^{65}Zn among the three size fractions (>3 μm, 3-0.2 μm, <0.2 μm) in the *Synechococcus* control (Fig. 2.3A) was relatively stable for the duration of the experiment. The EDTA pre-wash was effective in reducing the desorption of these isotopes from the radiolabeled *Synechococcus*. The EDTA pre-wash was less effective in the case of ^{153}Gd and ^{137}Cs; appreciable losses from the particulate phase were observed for both these isotopes during the 8-24-h phase of the experiments. Note, however, that these losses were enhanced in the presence of *Ochromonas* (*see below*).

The marked loss of both ^{153}Gd and ^{137}Cs from the *Synechococcus* cells in the prey-control treatments is surprising given their very different chemical properties - dissimilar loss mechanisms are likely involved. The loss of ^{137}Cs appeared to be due to a diffusion controlled loss from within the cyanobacterial cells as observed during an experiment using a higher concentration of ^{137}Cs (Expt. 3). In the case of ^{153}Gd, a loss from the picoplankton (3-0.2 μm) size fraction in control cultures in all replicates of this experiment was consistently observed. To test the cause of this loss, *Synechococcus* was pre-labeled with ^{153}Gd and ^{65}Zn, and then it was exposed to $10^{-4}M$ EDTA for various amounts of time (up to 106 min) in order to follow the loss rates of these radionuclides from the *Synechococcus* biomass. A significant amount of ^{153}Gd occupied binding sites on the cell surface and rapidly exchanged with EDTA within the first 7 min of the EDTA exposure (Appendix A). However, slow loss of ^{153}Gd continued to occur after this initial rapid exchange, whereas the loss of adsorbed ^{65}Zn in this test was complete within 7 min. The slowly exchanging Gd fraction presumably corresponds to non-labile Gd that is bound to the cell surface (slow kinetics of cell ligand-Gd dissociation) or reflects physical impediment of Gd diffusion from occupied ligands on the cell surface (e.g. retention within the crystalline s-layer of the cell surface, Schultze-Lam et al. 1992).

The slight accumulation of radioactivity on the 3-μm filter for the prey-control flasks is inferred to have resulted from the collection on the filter of clumped or dividing *Synechococcus* cells. This particular strain of *Synechococcus* is cylindrical and cells undergoing division or approaching it are rather elongated and some could have been retained by a 3-μm filter; supporting this notion, a decrease in the amount of radioactivity collected for all metals on the 3-μm filter correlated with a decrease in growth rate in the prey-control treatment (Fig. 2.2, *Synechococcus* alone). Adsorption of dissolved metals by filters was slight (maximum <2.5%), based on controls consisting of treatment filtrate (<0.2 μm) re-filtered through the various filters and rinsed as usual.

2.4.5 Metal partitioning between the particulate and dissolved phases: Grazing treatment

In the treatment containing *Synechococcus* and *Ochromonas*, a significant transfer of ^{109}Cd, ^{65}Zn and ^{153}Gd into the >3-μm size fraction (Fig. 2.3B) occurred. This accumulation corresponded to a *decrease* in the radionuclide content of the 3-0.2-μm size fraction (Fig. 2.3B) and a measured *decrease* in *Synechococcus* cell density (Fig. 2.2). Concurrently, the concentrations of all radionuclides in the dissolved phase (<0.2 μm) in this treatment increased relative to the concentrations observed in the prey

control. This evidence strongly suggests that some portion of the radionuclides added in particulate form, associated with *Synechococcus*, was transformed into a dissolved form as a result of the grazing by *Ochromonas*. A fraction of the consumed metal remained in the *Ochromonas* biomass but the majority of all consumed radionuclides was regenerated (Table 2.3). This behaviour was consistently observed in three independent repetitions of this experiment; figures from one experiment (Expt. 1; Figs. 2.2 and 2.3) are presented and the results of all experiments are summarized in Table 2.3.

The losses due to washes (Table 2.3) may be large due to the presence of amorphous material, originating from *Ochromonas* digestion, which has been observed with light microscopy. Such material is likely only loosely attached to cells and is apt to be removed during the wash step.

2.4.6 Short-term regeneration of radionuclides by grazing activity

Determining the degree of radionuclide regeneration efficiency by *Ochromonas* in the grazing treatment is complicated by the grazing-induced changes in particle loadings. Hence, we have chosen to estimate the degree of regeneration over the initial 9-16 h, to minimize the differences in particle loading between the prey control and grazing treatments. Although significant changes in particle loading might be expected to affect the partitioning of surface-bound radionuclides between the particulate and dissolved phase, with the exception of ^{153}Gd and ^{137}Cs, no change in the radionuclide cell quota of *Synechococcus* occurred over this time period. The estimates of short-term regeneration (Fig. 2.4) were calculated for this initial exposure period, which corresponded to 28 ± 7% of the entire assay duration, and during which the *Synechococcus* cell densities in the grazing treatment were 62 ± 6% of those in the prey control (values are the mean ± SD of the three experiments).

For trace metals primarily internalized within the prey cell, the degree of regeneration (Cd > Zn; Fig. 2.4) appeared to be inversely related to their respective first hydrolysis constants (Table 2.2). The regeneration efficiency of *Ochromonas* grazing showed a correlation with grazing rate for Cd, Zn, and Gd (Fig. 2.4). The data suggest an inefficient transfer of these trace metals via consumptive routes. Cesium data were limited to results from Expt. 3; in this experiment a high ^{137}Cs concentration was used in order to obtain reliable estimates of short-term regeneration. These estimates for trace metal regeneration are considered to be minimum values, in that some portion of the radionuclides that are ingested with the picoplankton, and subsequently regenerated into the dissolved phase, will not remain in solution but rather will sorb to available surfaces (both algal cell surfaces and container walls). Such resorption would lead to an underestimate of the true regeneration efficiency of *Ochromonas* grazing

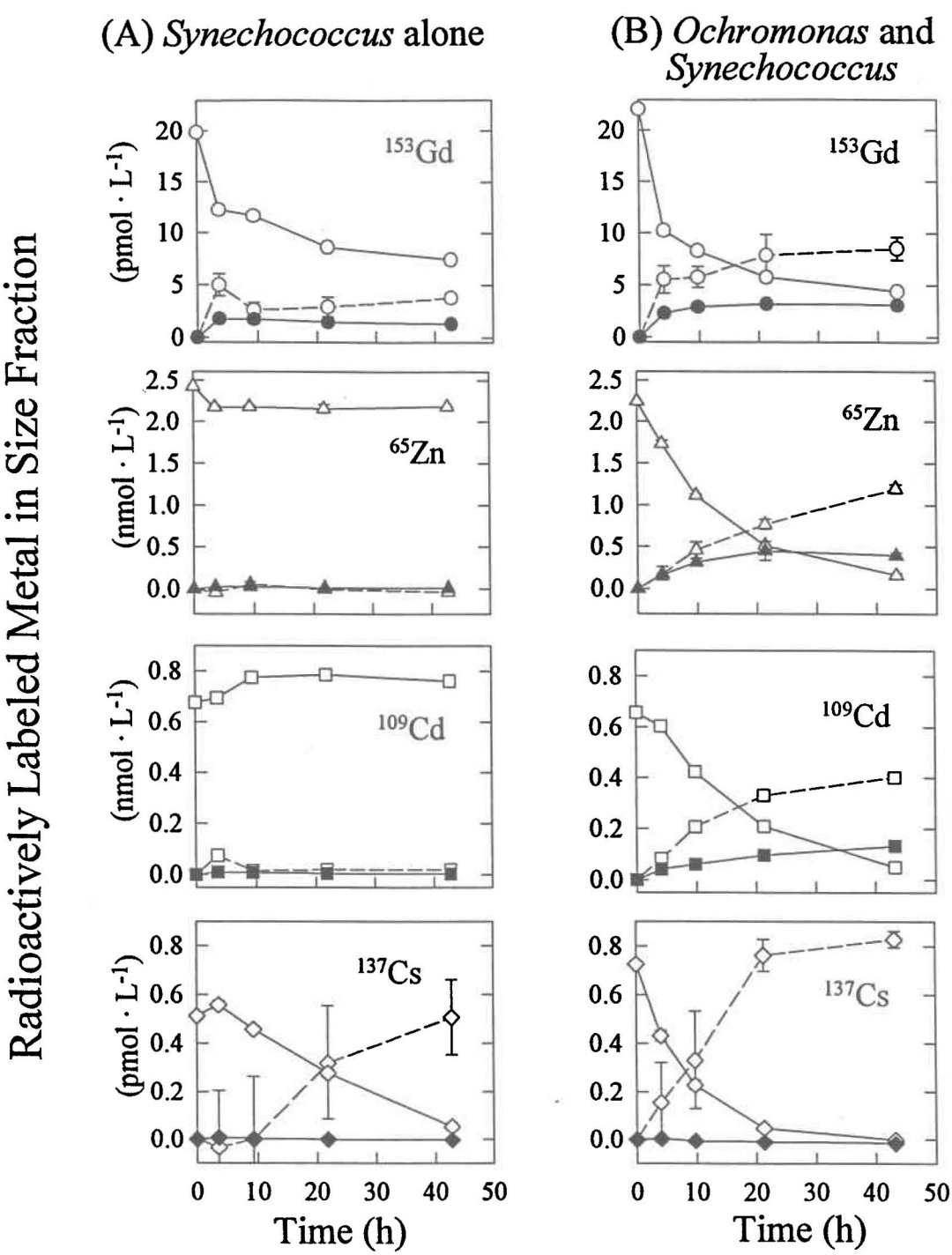

Figure 2.3. Partitioning of radionuclides among two particle size classes (>3 μm, 3-0.2 μm) and the dissolved phase (<0.2 μm) from Experiment 1. Radionuclides were added as a particulate phase (3-0.2 μm) in the form of radiolabeled cells of *Synechococcus* to both prey control (*Synechococcus* alone) or grazing (*Synechococcus* and *Ochromonas*) treatments. Size fractions: >3 μm (solid symbols); 3-0.2 μm (hollow symbols with solid line); <0.2 μm (hollow symbols with dashed line). Values are mean ± SD, *n* = 3; error bars are shown only when larger than the size of the symbol.

Table 2.3. Experimental summary of the mass balance of radionuclide partitioning among size fractions in the grazing treatments. Values for partitioning among size fractions and losses at the end of the experiment are percentages of the initial radioactivity (added in the form of radiolabeled *Synechococcus*) in the treatment at t_0. Sorptive losses to culture vessel were determined as the change in total aqueous radioactivity over the course of the experiment. Losses of radioactivity from particles collected on filters and washed were calculated as 100 - Σ all other fractions. Values are mean ± propagated SD.

	^{153}Gd	^{65}Zn	^{109}Cd	^{137}Cs
Exp. 1: 4% of initial *Synechococcus* population density remained after 43 h.				
Size fraction:				
>3 μm (*Ochromonas*)	13 ± 2.0	17 ± 1.4	19 ± 1.4	---
3-0.2 μm (*Synechococcus*)	19 ± 2.6	6.9 ± 1.0	7.1 ± 1.0	---
<0.2 μm (dissolved)	36 ± 6.8	51 ± 2.2	57 ± 1.1	>100
Sorbed to culture vessel	2.5 ± 14	8.7 ± 0.8	5.2 ± 1.8	---
Loss due to washes	29 ± 16	17 ± 2.9	12 ± 2.8	---
Exp. 2: 10% of initial *Synechococcus* population density remained after 45 h.				
Size fraction:				
>3 μm (*Ochromonas*)	13 ± 1.9	30 ± 11	24 ± 9.0	<1
3-0.2 μm (*Synechococcus*)	16 ± 1.7	12 ± 2.2	9.2 ± 2.5	1.2 ± 2.2
<0.2 μm (dissolved)	35 ± 3.6	36 ± 2.2	47 ± 2.1	58 ± 18
Sorbed to culture vessel	36 ± 3.9	<1	3.6 ± 0.8	23 ± 29
Loss due to washes	0.8 ± 5.8	23 ± 12	16 ± 10	20 ± 34
Exp. 3: 37% of initial *Synechococcus* population density remained after 49 h.				
Size fraction:				
>3 μm (*Ochromonas*)	16 ± 0.7	21 ± 3.1	18 ± 2.4	0.3 ± 0.1
3-0.2 μm (*Synechococcus*)	19 ± 1.4	17 ± 0.9	18 ± 1.2	0.7 ± 0.1
<0.2 μm (dissolved)	32 ± 4.7	30 ± 1.4	46 ± 2.6	88 ± 9.0
Sorbed to culture vessel	18 ± 6.3	3.6 ± 4.3	2.8 ± 2.5	3.7 ± 9.9
Loss due to washes	15 ± 8.1	29 ± 5.5	15 ± 4.5	7.1 ± 13

activity–this phenomenon would be expected to be more important for Gd, with its high affinity for surfaces, than for the other radionuclides.

2.4.7 Total radionuclide concentrations

With the exception of ^{153}Gd, total radionuclide concentrations varied little over the course of the experiment and only significantly by the end of the experiment in the grazing treatment (Table 2.3). The losses that did occur were attributed to sorption of radionuclides from the dissolved phase to the flask walls. Adherence to flask walls of dissolved and particulate organic carbon originating from

exocytosis might have contributed to the enhanced loss of radionuclides from the dissolved phase in the grazing treatments; such compounds would contain radionuclides and trace metal complexing ligands.

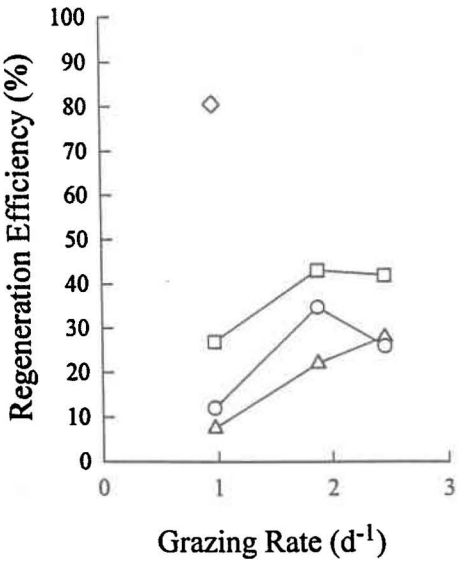

Figure 2.4. Degree of regeneration of ^{137}Cs, ^{109}Cd, ^{65}Zn and ^{153}Gd from *Synechococcus* by *Ochromonas* grazing over a range of specific grazing rates (see text for an explanation of the method used to calculate regeneration efficiency). Symbols: O–^{153}Gd; Δ–^{65}Zn; □–^{109}Cd; ◊–^{137}Cs.

2.4.8 Fractionation of the <0.2 μm filtrate

Early in the experiment (4 h), similar amounts of each radionuclide were observed in the <0.2-μm and <5-kD filtrates in the prey-control and grazing treatments (Fig. 2.5A, C). Most metal passing the 0.2-μm filter after 4 h in both treatments also passed the 5 kD-ultrafilter.

After 43 h, dissolved ^{109}Cd concentrations in the prey control (Fig. 2.5B) were less than those observed after 4 h (Fig. 2.5A). This reduction is attributed to resorption of ^{109}Cd, initially desorbed from the inoculum, by new cells. In the grazing treatment, a marked increase in dissolved ^{109}Cd and ^{65}Zn concentrations was observed after 43 h (Fig. 2.5D), as mentioned earlier. Most of the dissolved ^{109}Cd (75%) was of an apparent molecular mass <5 kD whereas only 10% of the dissolved ^{65}Zn was of a size <5 kD. Dissolved (<0.2 μm) concentrations of ^{153}Gd and ^{137}Cs were greater after 43 h compared with 4 h but the differences, although significant, were not as great as those observed for the two divalent cations. Repetitions of this fractionation at the termination of the experimental replications gave the following distributions (% filtrate < 5 kD), for Expts. 1, 2, and 3, respectively): ^{153}Gd: 74, ---, ---; ^{65}Zn: 10, 16, 49; ^{109}Cd: 75, 90, 54; ^{137}Cs: 37, 100, 100. Overall, these size separations suggest that

regenerated Cd was present in smaller chemical species than was regenerated Zn, and that dissolved Cs and Gd chemical species were generally less than 5 kD. Propagated counting errors for ^{109}Cd (Expts. 2 and 3) were <15% (P <0.05). Values for ^{153}Gd in Expts. 2 and 3 were rejected due to apparent contamination (i.e. cpm in ultrafiltrate >100% of those in the 0.2-µm filtrate).

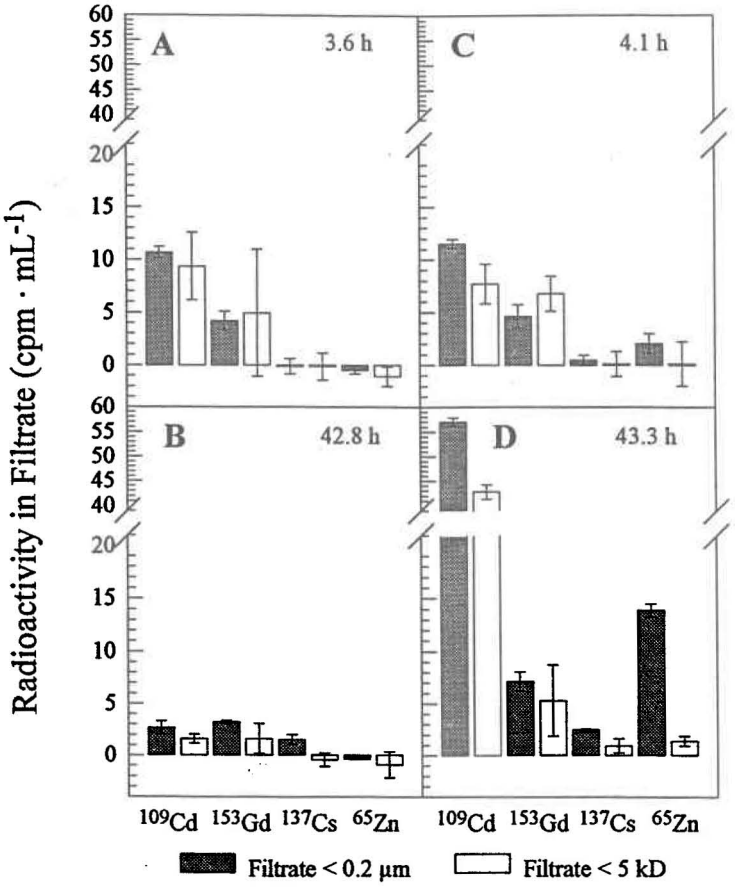

Figure 2.5. Fractionation of filtrate (<0.2 µm) from experimental treatments in Experiment 1 using ultrafiltration. A, B = *Synechococcus* alone (prey control); C, D = *Synechococcus* and *Ochromonas* (grazing treatment). Values are mean ± SD, n = 3.

2.4.9 Bioavailability of regenerated trace metals

Concentrations of individual radionuclides in the filtrate (<0.2 µm) from the grazing treatment were closely matched to those in a defined medium (Table 2.4). In a short-exposure experiment with these media and fresh inocula of *Synechococcus*, all radionuclides (except ^{137}Cs) were shown to be less

bioavailable in the <0.2-μm filtrate than in the fresh defined medium (Table 2.4). Neither total radioactivity nor cell density changed appreciably during the exposure period (5 h).

Table 2.4. Accumulation of radionuclides by *Synechococcus* at a concentration of 10^6 cells·mL^{-1} after 5 h in a radiolabeled inorganic defined medium (rFRAt) or radioactive filtrate (<0.2 μm) derived from a culture in which *Ochromonas* had grazed on radiolabeled *Synechococcus* over 49 h; media pH 7.5. Values are mean ± SD, $n = 2$.

		^{153}Gd	^{65}Zn	^{109}Cd	^{137}Cs
		Total radioactivity (cpm·mL^{-1})			
rFRAt	Mean	1.8	4.1	12.4	28.5
	SD	0.8	0.2	0.0	0.1
Filtrate	Mean	3.9	7.1	10.9	26.6
	SD	0.1	0.1	0.1	0.1
		Bioaccumulated metal (cpm·mL^{-1})			
rFRAt	Mean	1.055	0.310	0.481	0.004
	SD	0.041	0.018	0.042	0.003
Bioavailability[a]		59	7.6	3.9	0.01
Filtrate	Mean	0.455	0.158	0.147	0.047
	SD	0.081	0.013	0.043	0.106
Bioavailability[a]		12	2.4	1.3	0.18

Note: [a]Bioaccumulated radionuclides as percentage total.

2.5 DISCUSSION

2.5.1 Trace metal regeneration by micrograzing activity

These data demonstrate that grazing of picoplankton by nanoflagellates can effectively convert particulate trace metals into dissolved forms. The mixotrophic nanoflagellate *Ochromonas* consumed the picocyanobacterium *Synechococcus* by phagocytosis and the appearance of radionuclides in the dissolved phase was directly related to this grazing activity. This observation provides direct and convincing proof that through its consumption and digestion of picoplankton, *Ochromonas* regenerates a significant fraction of the trace metals that it consumes. The rapid appearance of dissolved Cd and Zn in the presence of grazing activity is particularly striking, in that most (>93%) of the Cd and Zn was

internalized within the algal prey, as shown by the EDTA extraction results. Whereas little desorption of these metals occurred from *Synechococcus* in the control treatment, significant amounts of Cd and Zn were observed in the dissolved phase in the grazing treatment. Clearly, the digestive strategies of nanoflagellates are capable of efficiently releasing into the dissolved phase trace metals previously internalized by prey cells.

To explain the partitioning of radioactive iron among various sized particulates, after the addition of Fe-radiolabeled *Synechococcus* to natural marine waters, Hutchins et al. (1993) hypothesized that microzooplankton were acting to recycle Fe in marine environments. Further work indicated that protozoa may regenerate Fe from their prey (Hutchins and Bruland 1994) but potential filtration artifacts precluded an unequivocal demonstration of regeneration. In the present study, by observing a nanoflagellate protozoan in a model system, it was experimentally confirmed that a suite of trace metals can in fact be regenerated through microzooplankton grazing activity. The trace metals used demonstrate that both nutrient metals (Zn) as well as non-nutritive metals (Gd, Cd, Cs) are regenerated by this grazing activity; these findings support the earlier results (Hutchins et al. 1993, Hutchins and Bruland 1994, 1995) which suggested that protozoa could regenerate Fe.

2.5.2 Consideration of potential methodological artifacts

Microscopic observation of the <3-μm filtrate in the grazing treatment (Expt. 1) at 43 h revealed a filtration efficiency of 87% (i.e. 87% of the *Ochromonas* were removed). Of the *Ochromonas* cells traversing the 3-μm filter (13% of total) approximately 70% lysed, as determined by a tally of free chrysolaminarin vesicles using light microscopy. A loss of *Ochromonas* would underestimate the radionuclides actually accumulated in the >3-μm size fraction, and would contribute to radioactivity found in the 3-0.2-μm and <0.2-μm fractions. However, any contribution to the dissolved (<0.2 μm) phase by lysing *Ochromonas* would be much less than that due to regeneration by exocytosis. In addition, the gravity filtration technique used here was compared with reverse filtration (Nagata and Kirchman 1990) to test whether the former technique might be provoking cell lysis; rupture of the protozoan cells on the filter might lead to an increase in the radionuclide content of the filtrate. In fact, no significant difference (P<0.001) was found between the two filtration approaches (Appendix B). Hence, it is concluded that the appearance of dissolved radionuclides in the <0.2-μm filtrate was not due to a filtration artifact.

Lysis of *Ochromonas* during the initial filtration step could have contributed colloidal organic material to the dissolved phase and thus affected the size fractionation of trace metals in the <0.2-μm filtrate. Based on the results of Tranvik (1994), however, this possibility is discounted. Tranvik concluded that cell lysis of the chrysophyte *Poterioochromonas malhamensis* during filtration contributed relatively little to filtrate dissolved organic matter compared to that arising from the protozoan grazing on bacterial prey. In the present case, if cell lysis of some *Ochromonas* had caused significant ligand production (>5 kD), then a difference in the partitioning of radionuclides within the <0.2-μm filtrate would have been expected between control and grazing treatments at $t \approx 4$ h (Fig. 2.5A, C). Since this was not the case, it is concluded that lysis of *Ochromonas* did not greatly alter the size distribution of the radionuclides in the dissolved phase.

2.5.3 Mechanisms of trace metal regeneration by microzooplankton

Trace metals bound by picoplankton could be regenerated by microzooplankton by several mechanisms. Intracellular digestion of prey cells in digestive vacuoles is thought to proceed by enzymatic degradation at reduced pH followed by alkalinization of the vacuole prior to its exocytosis as a vesicle containing waste products (*cf.* Capriuolo 1990). Metals adsorbed onto prey surfaces would be rapidly released from surface binding sites at the reduced pH of the digestive vacuole whereas internalized metals might be solubilized upon enzymatic degradation of the prey cell structure. During the subsequent alkalinization of the vacuolar sap, competition among organic ligands and the ^{-}OH ligand for complexation of hydrolysable metals would occur. The result of this ligand competition would control the form of the metal released upon exocytosis.

In preliminary grazing experiments it was observed that 2-μm-sized particles were produced by grazing activity. During direct microscopic observations of living *Ochromonas* actively grazing on *Synechococcus*, it was noticed that during mitotic division of *Ochromonas* the secondary digestion vacuole (the largest) is not shared between the sister cells but is instead ejected from the dividing cell. This vacuole (≈2 μm-diameter) is a lipid-bound vesicle containing partially digested material. The interior of digestive vacuoles in protozoa has been found to be acidic (pH 2; Capriulo 1990) but the proton gradient is expected to degrade rapidly upon expulsion of the vacuole, causing lysis and a release of the material held within.

2.5.4 Trophic transfer of trace metals

Radionuclides accumulated in the *Ochromonas* size fraction may have been present as radiolabeled prey in the digestive vacuole, as radionuclides transferred to the consumer during the digestive process, or as metal sorbed from the dissolved phase by *Ochromonas* cells. *Ochromonas* accumulated less ^{153}Gd than ^{109}Cd and ^{65}Zn, relative to the initial amount of radionuclides present. Also, there was a consistent enrichment of ^{153}Gd in the 3-0.2-μm size fraction in the grazing treatment relative to ^{109}Cd and ^{65}Zn, despite the desorptive losses of the rare earth element evident in the prey-control treatment. This suggests that the regenerated ^{153}Gd may have had a greater affinity for cells and debris (3-0.2 μm) than ^{109}Cd and ^{65}Zn, as would be expected based on its greater surface reactivity. Accordingly, the contribution of sorption from the dissolved phase to overall metal accumulation in the *Ochromonas*-size fraction was probably greater for ^{153}Gd than for ^{109}Cd or ^{65}Zn.

Reinfelder and Fisher (1991) show a strong correlation between assimilation efficiency of several trace metals by copepods and the cytoplasmic content of these elements in diatom prey. This relationship may also be relevant for microzooplankton. Fisher et al. (1995) observed an accumulation of ^{110m}Ag, but not ^{210}Pb, in the ciliate *Fabrea salina* feeding on radiolabeled diatom prey. Like Gd, Pb is primarily sorbed to the cell surface whereas Ag, like Cd and Zn, is internalized by algal cells. If nanoflagellates have the same "liquid" digestive strategy as copepods (Reinfelder and Fisher 1991) and bivalve larvae (Reinfelder and Fisher 1994), then less ^{153}Gd than ^{109}Cd or ^{65}Zn would be expected to be accumulated through digestion by *Ochromonas*.

2.5.5 Ecological relevance of laboratory experiments to the Laurentian Great Lakes

Both of the organisms chosen for this study are key members of the microbial food web in the Laurentian Great Lakes. In Lakes Michigan and Huron, the genus *Synechococcus* accounts for as much as 26% of the primary production and *Ochromonas* is one of the dominant members of the protozoan community that controls *Synechococcus* populations through intense grazing (Fahnenstiel et al. 1991a). Concentrations of organisms used in the laboratory grazing experiments were 2 to 6 times greater than maximum values observed in the lower Laurentian Great Lakes. Observed maxima for photosynthetic picoplankton cell densities are 7.5×10^8 cells·L^{-1} (Lake Ontario, Caron et al. 1985) and 2.9×10^8 cells·L^{-1} (Lake Erie, Weisse and Munawar 1989), whereas observed maxima for heterotrophic nanoflagellates are 1.2×10^6 cells·L^{-1} (Lake Ontario, Caron et al. 1985) and 3.1×10^6 cells·L^{-1} (Lake Erie, Weisse and Munawar 1989).

The cell densities of *Synechococcus* and *Ochromonas* used in the experiments gave particulate organic carbon (POC) concentrations somewhat higher than those observed for plankton (<20 μm) in Lake Ontario during peaks in picoplankton abundance during summer months (Caron et al. 1985). This estimate is based on observed cyanobacterial picoplankton densities of 6.5×10^8 cells·L^{-1} (10% of the bacterial picoplankton densities), assumed cell diameters of 0.8 μm for cyanobacteria and 0.3 μm for bacteria, 106 fg C·μm^{-3} for cyanobacteria (Nagata 1986) and 220 fg C·μm^{-3} for bacteria (Bratbak and Dundas 1984). Caron et al. (1985) found that the picoplankton in Lake Ontario comprised 50% of the plankton biomass <20 μm at the time of their sampling; the POC attributable to the picoplankton (0.2-2 μm) and the nanoplankton (2-20 μm) at this time would be 80 μg POC·L^{-1}. This POC content is a sixth that of the 465 μg POC·L^{-1} in the grazing treatments in the present study, estimated using a conversion factor of 106 fg C·μm^{-3} (cell diameters: *Synechococcus* = 1.5 μm, *Ochromonas* = 5.5 μm).

Metal loadings in the treatments (added as particulate metal in the form of radiolabeled *Synechococcus*) were within an order of magnitude of dissolved metal concentrations measured in the pelagic zones of Lakes Erie and Ontario (Cd and Zn) and those found in the open ocean (Gd) (Table 2.1). Values for Gd concentrations in the Laurentian Great Lakes (pH 7.5 - pH 8.5) are presently unknown although dissolved Gd has been found in the range 55-500 pM in river water (pH 7.0-7.5) from the United Kingdom (Elderfield et al. 1990). Based on a direct measurement of dissolved La (51 pM) and a Gd:La shale mole ratio of 1:7, a range of 5-15 pM for dissolved Gd has been estimated for Lake Champlain during the summer months (E.R. Sholkovitz, pers. comm.); Lake Champlain is a large lake similar in ionic composition to the lower Laurentian Great Lakes.

2.5.6 Possible influences of micrograzing activity on trace metal bioavailability and residence times in surface waters

Particulate-bound metals (radiolabeled *Synechococcus*) were regenerated (through grazing activity by *Ochromonas*) into forms that were less available for resorption by picoplankton (*Synechococcus*) than were the same metals in equilibrium with the inorganic ligands present in the initial inorganic growth medium (Table 2.4). These results imply that, given similar particle loads and fluxes, regenerated trace metals will tend to accumulate to greater concentrations in the water column than would the same metals introduced in dissolved inorganic form (e.g. from the atmosphere)—this

constitutes a direct biotic influence on elemental fractionation in the water column by microbial food web organisms.

Dissolved organic matter produced by nanoflagellate grazing activity is more recalcitrant than dissolved organic matter excreted during autotrophic activity (Tranvik 1994). Organic ligands in dissolved or stable colloid form, originating from microzooplankton digestion, might thus serve as important trace metal buffers in the epilimnion. In this scenario, regenerated trace metals would be retained in the water column where they would be released and resorbed by particles. The ligands produced by microzooplankton grazing activity could complex trace metals upon excretion and contain complexed trace metals originating directly from digested prey.

The "classical" picture of dissolved trace metal concentrations in surface waters being controlled by sorption complexation between soluble and particulate ligands appears to be incomplete. The flux of particulate metals to the sediment ultimately controls their dissolved concentration, but more is involved than surface complexation equilibria. The present experiments demonstrate that biotic activity, microzooplankton grazing in particular, may affect elemental fractionation with each re-enactment of the digestive processing of trace metals scavenged by picoplankton.

CHAPTER THREE

REGENERATION, RECYCLING, AND TROPHIC TRANSFER OF TRACE METALS BY MICROBIAL FOOD WEB ORGANISMS IN THE PELAGIC SURFACE WATERS OF LAKE ERIE[1]

3.1 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 measure grazing activity independently. The majority of 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 2.9 and 2.5 times more efficiently transferred than ^{109}Cd into the microplankton and nanoplankton, respectively. 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 kD; 8 ± 24% ^{65}Zn <5 kD). These results show that microzooplankton grazing will tend to prolong the residence times of such metals as Cd and Zn in the pelagic surface waters of large lakes.

3.2 INTRODUCTION

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. 3.1A). Metals may become associated with this particulate matter by various sorptive processes (scavenging) such as adsorption, co- precipitation, and in the case of biological organisms, cellular internalization. Various inorganic surfaces, such as calcite, clays, and iron and manganese oxyhydroxides, may be involved in scavenging but in the pelagic

[1] Twiss, M.R., Campbell, P.G.C., and Auclair, J.-C. 1996. Regeneration, recycling, and trophic transfer of trace metals by microbial food web organisms in the pelagic surface waters of Lake Erie. Limnol. Oceanogr. 41: (in press).

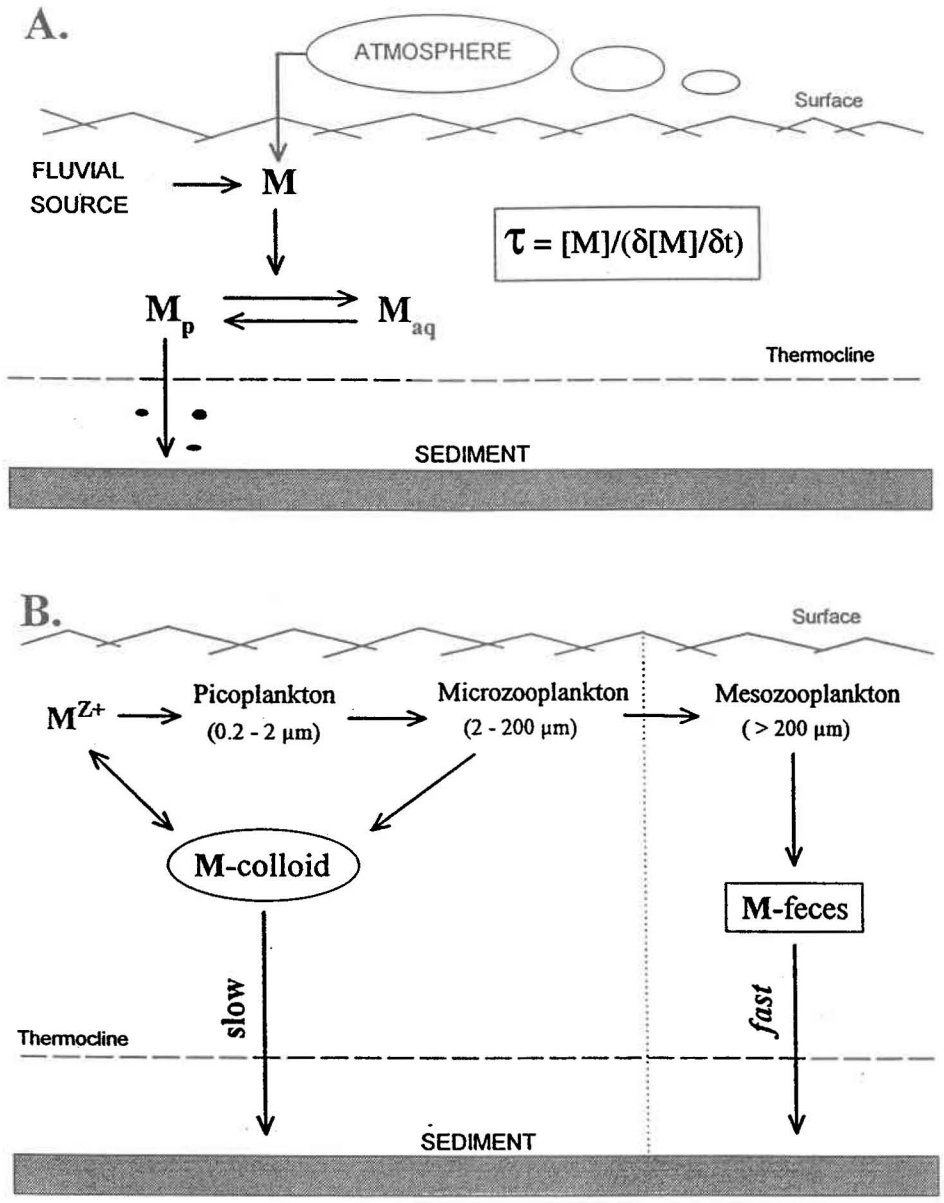

Figure 3.1A-B. The biogeochemical fate of trace metals in the pelagic region of large lakes during thermal stratification. **A.** Physical and chemical influences. Trace metals enter the water column in particulate or dissolved form and partition among dissolved (M_{aq}) and particulate (M_p) phases. The flux of particulate metal to the sediment controls the residence time (τ) of trace metals in the surface waters. **B.** Biological influences. The majority of 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 thus 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.

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 scavenging trace metals (Fisher 1985) due 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). Since the ecological fate of the picoplankton in the Laurentian Great Lakes is largely determined by microzooplankton grazing (Fahnenstiel et al. 1986, 1991a) it follows that trace metals scavenged by picoplankton will also be affected by this activity (Fig. 3.1B).

Hutchins et al. (1993), in a study of water sampled from the equatorial Pacific Ocean, inferred that microzooplankton were regenerating iron from Fe-radiolabeled picoplanktonic cyanobacteria into the dissolved phase, thereby increasing its bioavailability 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 (*cf.* Chapter Two). The simplified microbial food web studied in Chapter Two involved two microorganisms which represent major contributors to the microbial food web of the Laurentian Great Lakes (Fahnenstiel et al. 1991a), viz. the mixotrophic nanoflagellate *Ochromonas* grazing the picocyanobacterium *Synechococcus*. Trace metal regeneration was tightly coupled to grazing activity. In the present study, we extended this hypothesis of trace metal regeneration by microzooplankton into the field, 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 various particle size fractions and the dissolved phase, showing how the geochemical cycles of trace metals and the ecological cycles of the plankton are linked in pelagic surface waters.

3.3 METHODS

3.3.1 Glassware Preparation

All plasticware and silanized glassware were rigorously cleaned. The cleaning protocol involved a warm soap wash (Liqui-Nox, 1%), methanol (HPLC grade) soak, HNO_3 (1.6 M, reagent grade) soak, HCl (0.8 M; Suprapur, Merck) soak with a 7-fold rinse after each cleaning step using deionized water ($\geq$17.5 Mohms·cm^{-1}).

3.3.2 Pre-exposure of Picoplankton to Trace Metal Radionuclides

A culture of the picocyanobacterium *Synechococcus leopoliensis* (UTEX 625) (referred to herein as *Synechococcus*) was exposed to the gamma-emitting radionuclides ^{137}Cs, ^{109}Cd and ^{65}Zn in modified Fraquil medium (rFRAt) at a cell density of $\approx 10^{10}$ cells·L^{-1}. Nominal metal concentrations (and radioactivities) were: ^{137}Cs, 5 nM (0.7 MBq·L^{-1}); ^{109}Cd, 5 nM (2.1 MBq·L^{-1}); ^{65}Zn, 10 nM (10.6 MBq·L^{-1}). After 40-60 h of exposure, these picoplankton were treated with 0.1 mM Na_2EDTA by adding an EDTA solution directly into the rFRAt. After 25 minutes, the cells were harvested onto 0.4-µm polycarbonate membrane filters (Nuclepore), rinsed with sterile lake water (*see below*) and resuspended in the same. This EDTA rinse technique has proven effective in removing loosely adsorbed trace metal radionuclides from the surface of *Synechococcus* (*cf.* 2.3.2). The radiolabeled picoplankton were then used to spike the natural plankton community present in sampled lake water.

3.3.3 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 Station 23 (Fig. 3.2) at 18h30 on July 11, 1994 using an acid-cleaned, Teflon-coated 8-L Go-Flo bottle (General Oceanics) suspended on a non-metallic line. Lake water was filtered through a 210-µm pore size polypropylene mesh (Spectrum); this water is referred to as "whole lake water". One 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) pre-purified 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 C.S.S. *Limnos*. Trace metal analysis of the sterile lake water prepared at Stn. 84 was conducted by G. Lawson of the National Water Research Institute, Burlington, ON. 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, comparable to measurements made in Lake Erie surface waters by other researchers using similar trace metal clean techniques (Coale and Flegal 1989, Nriagu et al. 1993, Nriagu et al. 1996).

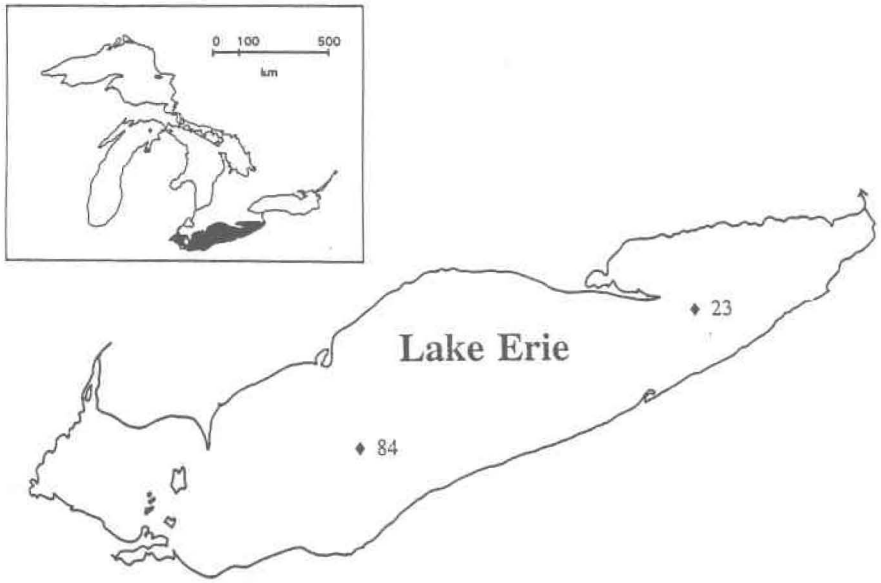

Figure 3.2. Map of the Laurentian Great Lakes showing the position of the study sites in the pelagic zone of the central and eastern basins of Lake Erie. Note: Station 23 is also known as Station 879.

Filtration efficiency of the Suporcap membrane filter was verified by comparing the fluorescence of the Suporcap filtrate (<0.2 μm), after passing ≈40 L 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), the fluorescence of filtered whole lake water (<0.2 μm, polycarbonate membrane filtration of 50 mL) was 0.05 FU whereas that of the Suporcap filtrate was only 0.03 FU. Phytoplankton were thus very efficiently removed by the Suporcap filter.

All water was retained in 20-L polycarbonate carboys prior to dispensing into test bottles through Tygon tubing using pressurized N_2. Experimental treatments were established by adding 2 L of water to 2-L polycarbonate bottles (Nalgene): the grazing treatment received whole lake water and the control treatment received sterile lake water. The whole lake 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·L^{-1}. Bottles were incubated

in an incubation chamber simulating *in situ* environmental conditions (20°C, 92 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 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 over 51 h. Partitioning of the 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 radioactivity retained by the filter. Likewise, 70 mL of the <20-μm filtrate were filtered onto a 3-μm polycarbonate filter (Nuclepore, 47 mm) and subsequently, 20 mL of the <3-μm filtrate were filtered onto a 0.2-μm polycarbonate filter (Nuclepore, 47 mm). In each case, the filtrate was removed before the filters were rinsed. Applied vacuum for filtration by the 3-μm and 0.2-μm filters was <13 kPa. The final filtrate (<0.2 μm) was sampled (2 mL) in duplicate for radioactivity. In addition, duplicate 2-mL samples were removed at each sampling interval for determination of the total aqueous radioactivity in each bottle. The total volume of water removed from the sample bottles by the end of the incubation was less than 20%.

3.3.4 Assays for Measuring Grazing Rates and Growth Rates within the Microbial Community

Phytoplankton-specific grazing and growth rates were assessed using a 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-L 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.

3.3.5 Shortening the Microbial Food Chain

The purpose of this experiment was to assess the relative roles of nanoplanktonic and microplanktonic grazers in determining the fate of radiolabeled picoplankton. Water was collected at

13h30 on July 12, 1994 from a depth of 5 m at Station 84 (Fig. 3.2). 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 (*cf.* Section 3.3.2) and added to each bottle to give an initial density of 1.2 x 10^8 cells·L^{-1}. Bottles were incubated, with the accompanying dilution assay treatments, under simulated *in situ* conditions (20 °C, a 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.

3.3.6 Trapping regenerated trace metal using EDTA: a direct measurement of regeneration and recycling

Trace metals regenerated from radiolabeled-picoplankton by micrograzers will partition between the various particulate phases and the dissolved phase. The recycling of trace metals from the dissolved phase back into particles was measured 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 Station 23, with lake water collected at 16h00 on July 24, 1995 from a depth of 5 m. All manipulations were identical to those in the earlier experiment at Station 23 conducted on July 11, 1994 (as described above), with the exception that Na_2EDTA was added to one-half of the treatments; 3 control bottles and 3 grazing treatment bottles contained 2 μM EDTA, 3 control bottles and 3 grazing bottles received no EDTA.

Synechococcus was pretreated in rFRAt with ^{109}Cd, 1 nM (3.6 MBq·L^{-1}) and ^{65}Zn, 5 nM (10.7 MBq·L^{-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 x 10^7 cell·L^{-1}. Bottles for this experiment, and the accompanying dilution assay, were incubated together under simulated *in situ* conditions (20 ± 2°C, a light intensity of 150 μmol photons·m^{-2}·s^{-1} and a natural photoperiod). Bottles containing radioactivity were sampled at intervals over a 65 h period. Samples from the control bottles containing 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 kD ultrafilters; Ultraspin 8000, Lida Corp.) of the dissolved fraction (<0.2 μm) from each treatment replicate after 38 h. Ultrafiltration devices were pre-treated prior to use to minimize adsorptive losses of radionuclides, as described in Chapter Two (Section 2.3.3).

3.3.7 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 have chosen to use a 3-μm separation in our experiments with *S. leopoliensis* since this picocyanobacterium 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 if we used a 2-μm or 3-μm filter, despite the possibility that some small cryptomonad nanoflagellates might have squeezed through a 3-μm filter pore size 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.

3.3.8 Analytical Procedures

The concentration of chl-*a*, corrected for pheopigments, in samples 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 pre-exposure media and prey control treatments were determined using an electronic particle counter (Coulter Multisizer II; 15 μm orifice) on samples fixed with Lugol's Iodine (1.5%).

Radioactivity was determined with a gamma-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 990-1268 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 less than 10% ($P<0.05$).

Low ^{137}Cs radioactivity in samples required precise counting with a Ge(Li) detector (Canberra Instruments) 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 Station 23. Counting errors were <25% (P<0.05).

3.4 RESULTS AND DISCUSSION

3.4.1 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 the grazing treatments (<210 μm lake water). The disappearance of ^{109}Cd and ^{65}Zn from the size fraction containing the radiolabeled picoplankton spike (0.2-3 μm) was matched by the appearance of these radionuclides in the dissolved (<0.2 μm) phase (Fig. 3.3). In contrast, the amount of radionuclides desorbing from the radiolabeled *Synechococcus* in the control treatment was markedly lower.

In a general manner, both ^{65}Zn and ^{109}Cd behaved similarly, with the majority of the metal entering the dissolved fraction in the grazing treatments by the end of the experiment (Fig. 3.3; Fig. 3.4). For example, partitioning of ^{65}Zn in the whole lake water collected from Station 23 in July 1994 (Fig. 3.3) after 51 h was: 3%, 7%, 38% and 52%, for the microplankton, nanoplankton, picoplankton, and dissolved phase, respectively (values 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* (% 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. Approximately 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 measured in each fraction after 51 h in comparison with the measured total aqueous radioactivity at that time (Fig. 3.3). 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 due to sorptive losses to container walls; losses of total radioactivity were generally <5% for ^{65}Zn and <2% for ^{109}Cd.

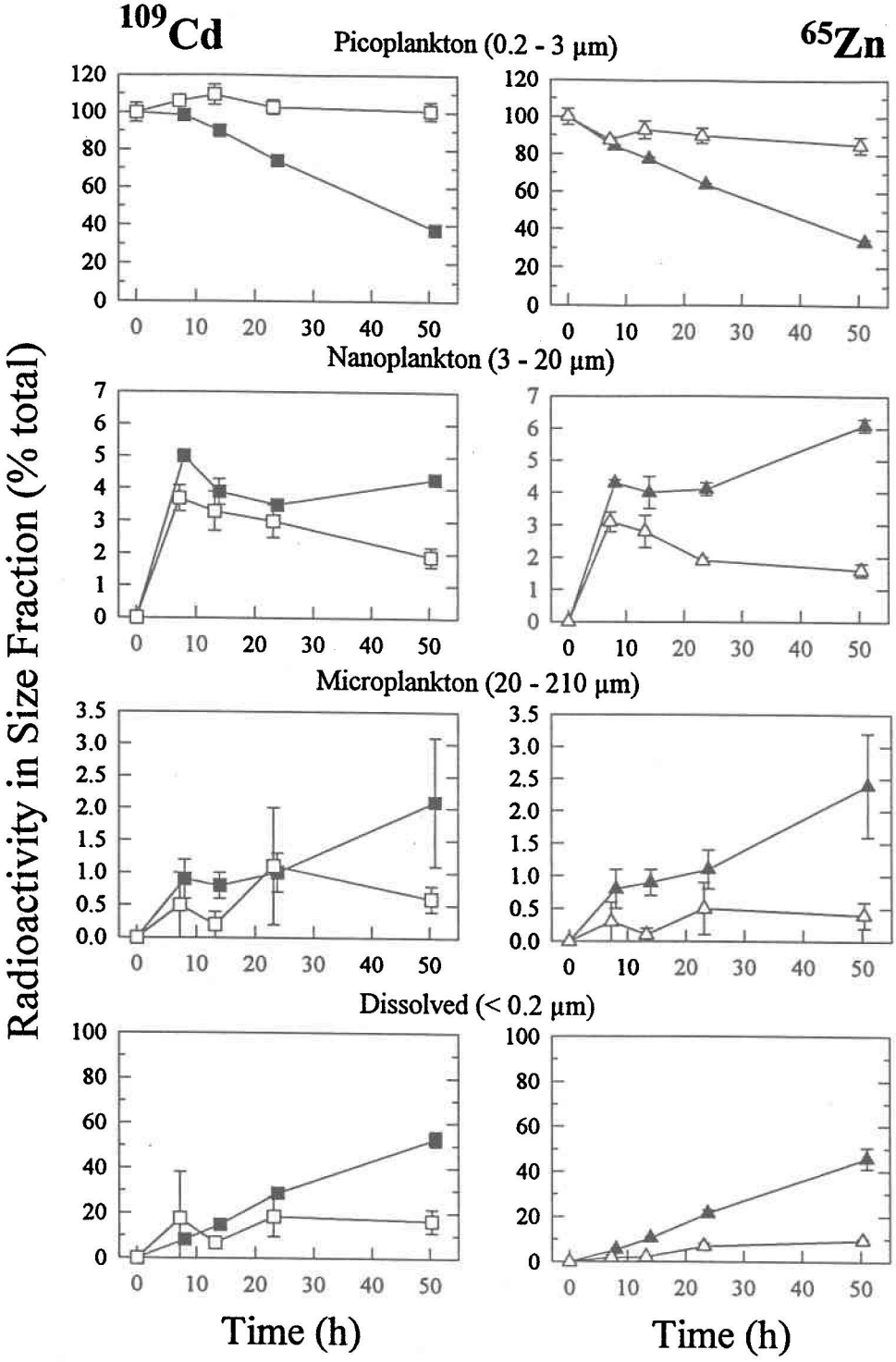

Figure 3.3. Partitioning of ^{109}Cd and ^{65}Zn among three planktonic size classes and the dissolved phase in grazing (whole lake water, <210 µm) and control (sterile lake water, <0.2 µm) treatments. Water was sampled from Station 23 on July 11, 1994. Radionuclides were added as radiolabeled cells 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 larger than the size of the symbol.

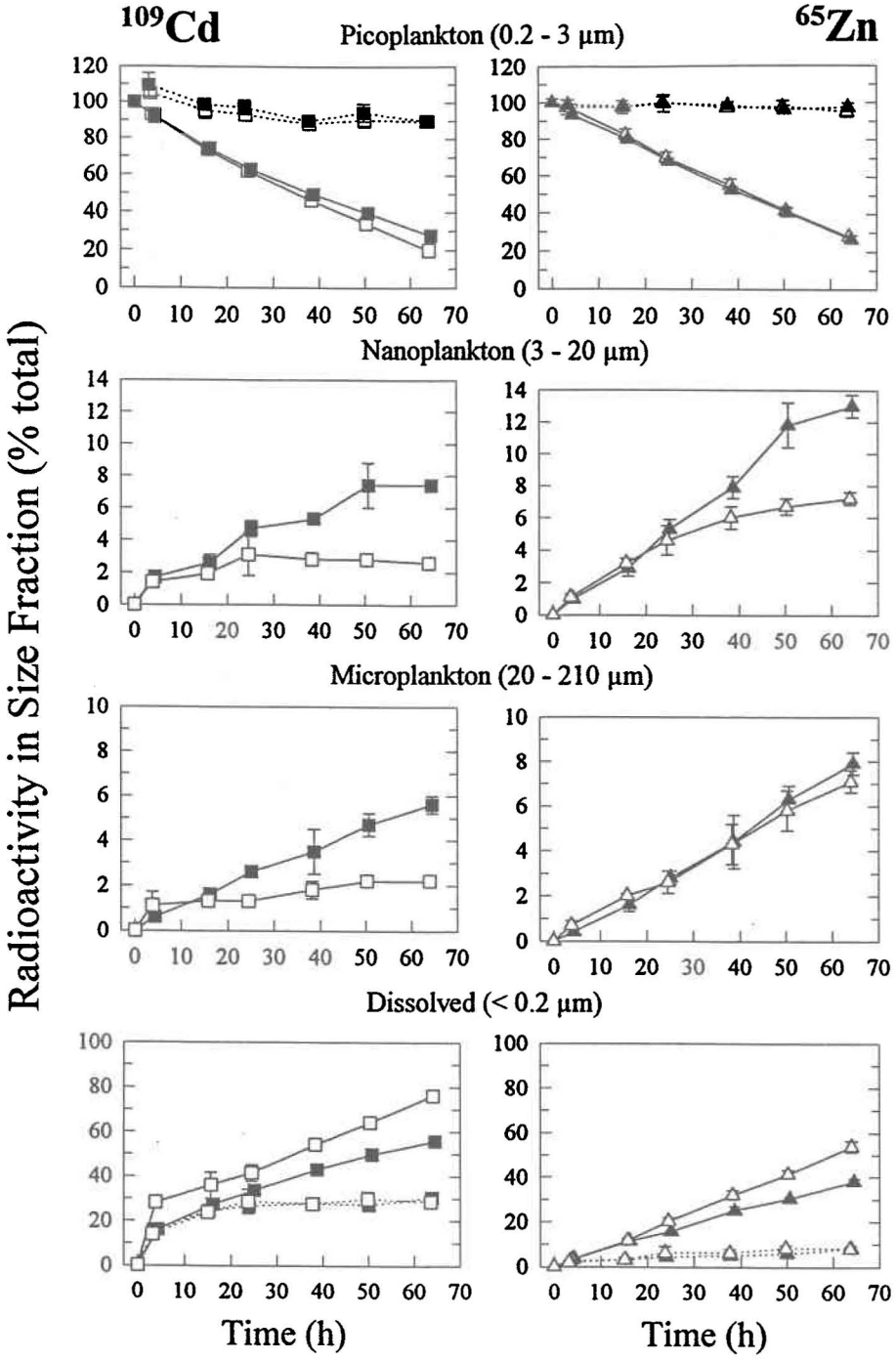

Figure 3.4. Partitioning of ^{109}Cd and ^{65}Zn among three planktonic size classes and the dissolved phase in grazing (whole lake water, <210 µm) and control (sterile lake water, <0.2 µm) treatments with and without added 2 µM EDTA. Water was sampled from Station 23 on July 24, 1995. Radionuclides 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 larger than the size of the symbol.

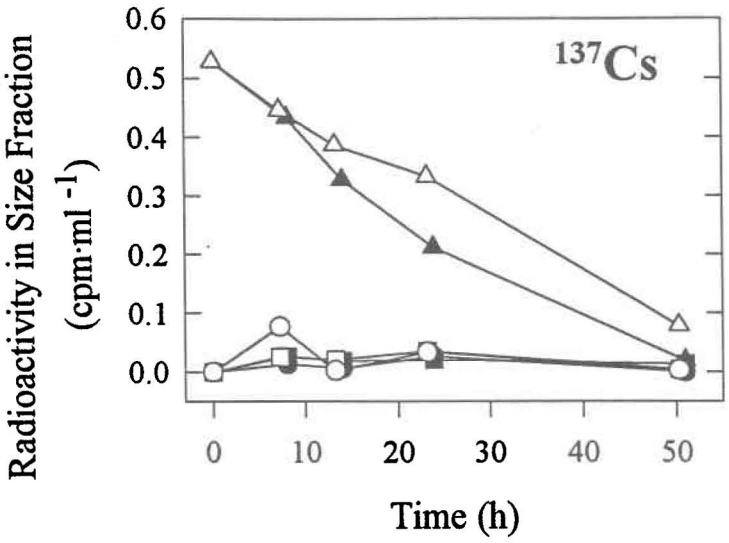

Figure 3.5. Partitioning of ^{137}Cs among three planktonic size classes in grazing and control treatments. Water was sampled from Station 23 on July 11, 1994. The control treatment (sterile lake water, <0.2 μm) is represented by hollow symbols; the grazing treatment (whole lake water, <210 μm) is represented by solid symbols. Size fractions: picoplankton, 3-0.2 μm (▲); nanoplankton, 20-3 μm (■); microplankton, 210-20 μm (•). No measurement of ^{137}Cs in the dissolved phase was made. Values are single measurements made from a single treatment replicate.

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. 3.5). A similar phenomenon was observed in laboratory experiments using the same prey organism in a defined, inorganic growth medium (*cf.* Chapter Two); the majority of the ^{137}Cs loss from the picoplankton cells is attributed to the diffusion of ^{137}Cs from intracellular pools.

3.4.2 Grazing of Picoplankton by Microzooplankton

Picoplankton comprised 31-36% of the phytoplankton biomass at the stations studied (Table 3.1). Dilution assays conducted on the same water samples with no added radiolabeled-*Synechococcus* showed high specific grazing rates of both picoplanktonic and nanoplanktonic chl-*a* in surface water sampled at both stations (Fig. 3.6). Repetition of the dilution assay at Station 84 on July 14, 1994, and Station 23 on September 6, 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*, respectively, during these sampling times. With the exception of the dilution assay conducted simultaneously with

the experiment at Station 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 3.2; Fig. 3.3; Fig. 3.4) 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.

Table 3.1. Characterization of the two pelagic study sites in Lake Erie.

	Station		
Parameter	23	23	**84**
Date	07-07-94	24-07-95	12-07-94
Depth of epilimnion (m)[a]	15	10	15
Total chlorophyll-*a* ($\mu g \cdot L^{-1}$)	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
Temperature at 5 m (°C)	21	22	21

Note: [a] Maximum depth at stations 23 and 84 was 60 m and 25 m, respectively.

3.4.3 Relative Importance of Nanoplanktonic and Microplanktonic Grazers

Both nanoplanktonic and microplanktonic grazers participated in the grazing of the added radiolabeled-*Synechococcus*. Observed specific 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 (Stn. 84; July 12, 1994):

Lake water	**Grazers Present**	**^{65}Zn**	**^{109}Cd**
<20 μm	nanoplanktonic	$0.08 \cdot d^{-1}$	$0.07 \cdot d^{-1}$
<210 μm	nanoplanktonic and microplanktonic	$0.13 \cdot d^{-1}$	$0.14 \cdot d^{-1}$.

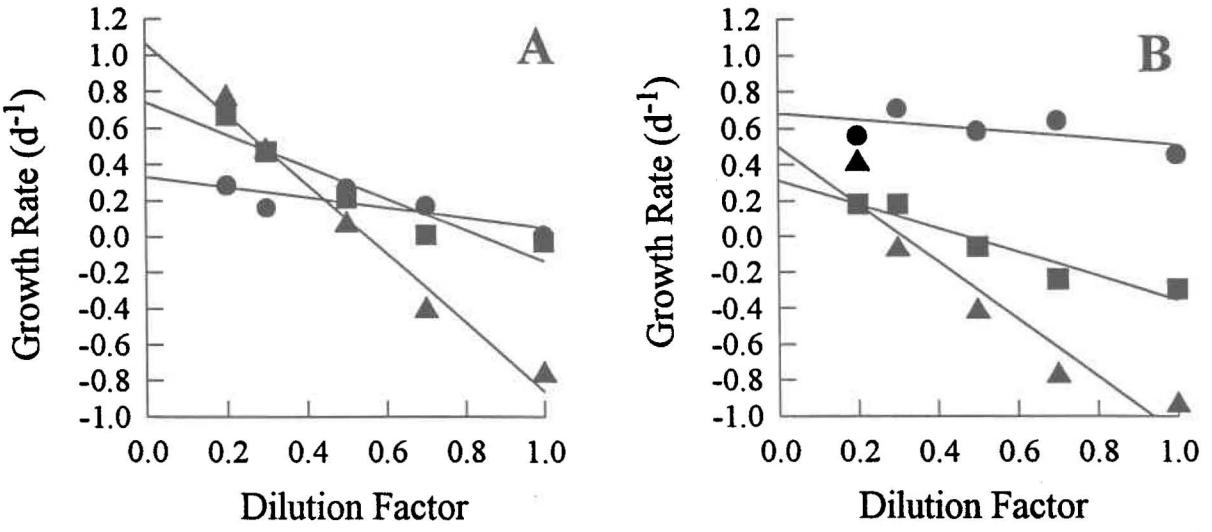

Figure 3.6. Results from dilution assays used to estimate the chlorophyll-*a* based specific rates of grazing and growth among the picoplankton (▲), nanoplankton (■) and microplankton (•) size classes from the eastern and central basin of Lake Erie. **A.** Station 23, eastern basin. **B.** Station 84, central basin. 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 specific growth rate versus dilution represents the specific grazing rate whereas the intercept is the estimated intrinsic specific growth rate. Observed chlorophyll-*a*-based growth (μ) and grazing (g) rates ($d^{-1} \pm$ SE) in each size fraction were: **A:** picoplankton, $\mu = 1.07 \pm 0.10$, $g = 1.93 \pm 0.16$; nanoplankton, $\mu = 0.74 \pm 0.12$, $g = 0.88 \pm 0.19$; microplankton, $\mu = 0.33 \pm 0.08$, $g = 0.28 \pm 0.12$; **B:** picoplankton, $\mu = 0.50 \pm 0.20$, $g = 1.60 \pm 0.32$, nanoplankton, $\mu = 0.31 \pm 0.08$, $g = 0.67 \pm 0.12$; microplankton, $\mu = 0.68 \pm 0.09$, $g = 0.18 \pm 0.13$.

This comparison of loss rates suggests 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 evidence from the literature suggests 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 near equal contributions to the grazing pressure on picoplankton by the nanoplanktonic and microplanktonic grazers. In accordance with this finding, Carrick et al. (1992) report 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 3.2). The relatively low ^{65}Zn- assimilation efficiency of the grazers in the microplankton (20-210 μm) compared to grazers in the nanoplankton (3-20 μm) might reflect different digestive strategies between organisms in these two groups. Grazers in the

Table 3.2. 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 Station 84 (July 12, 1994). All treatments received radiolabeled-*Synechococcus*. Filter sterilized lake water (<0.2 μm) was used to measure desorption of radionuclides from *Synechococcus*, filtered lake water (<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} ± standard deviation (n = 2); values in parentheses are filter controls. Values in brackets are percentage of the sum of the radioactivity measured in the fractions (this sum does not include radionuclide losses due to filter rinses).

		Lakewater Fraction		
Radio-nuclide	**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%]

nanoplankton size fraction appeared to regenerate more ^{65}Zn than those in the microplankton (Table 3.2). However, the reduced particle loading in the <20 μm treatment (microplankton comprised 8% of the chl-*a* in the whole lake water treatment; Table 3.1) 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 degree of ^{109}Cd loss from *Synechococcus* was observed in the prey control in this experiment (Table 3.2), effectively masking any regenerative processes. Some desorbed ^{109}Cd may have been re-adsorbed by particles in the grazing treatments, thereby giving no indication of any ^{109}Cd entering the dissolved phase by regenerative processes. A similar loss of ^{109}Cd, although less severe, was also observed in the experiment at Stn. 23 (1995; Fig. 3.4).

3.4.4 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 3.2; Fig. 3.3; Fig. 3.4). 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. 3.4). Since this experiment was conducted in the presence of an excess of 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 the 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 favourable intracellular partitioning in the picoplankton prey, compared with ^{109}Cd (*cf.* Reinfelder and Fisher 1990). 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 3.2 *and* Fig. 3.3), recycling rates were inferred by comparing the net specific loss rates of radioactivity in the picoplankton size fraction in grazing

treatments with specific loss rate of chl-*a* in the picoplankton size fraction obtained from dilution assays (Fig. 3.6). 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 the 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

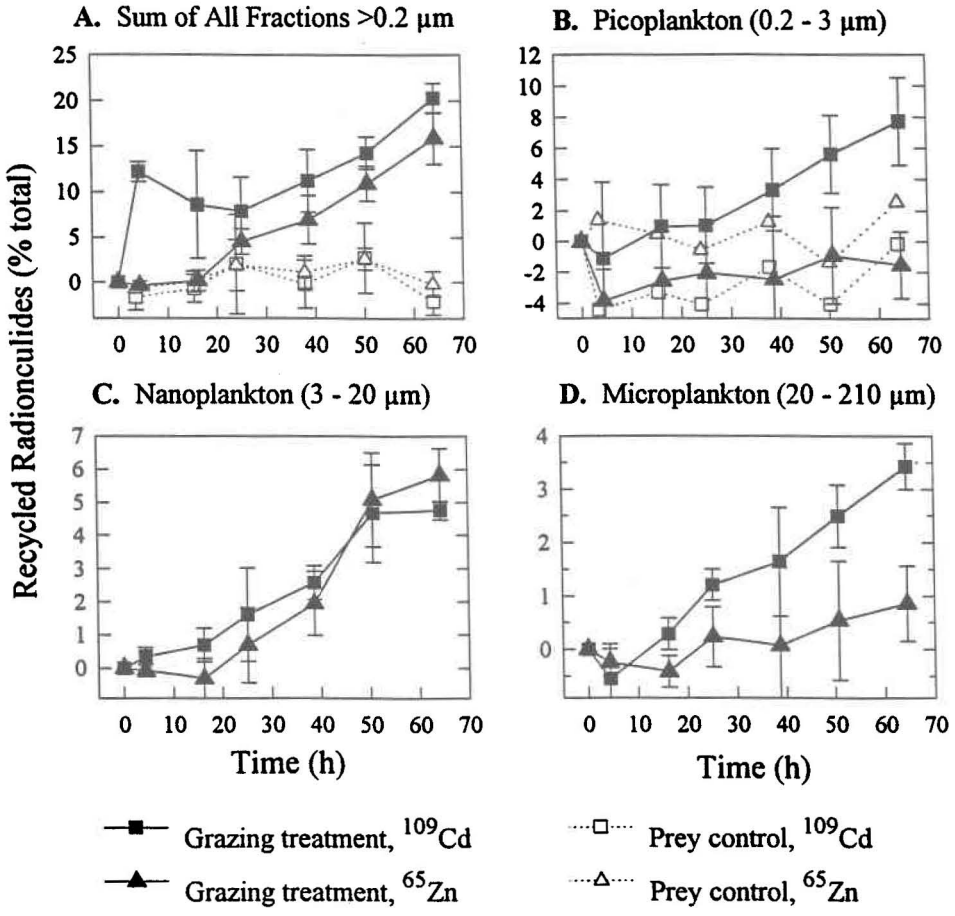

Figure 3.7. Estimated recycling of ^{109}Cd and ^{65}Zn from the dissolved phase by various plankton fractions. Recycling was calculated by the difference in radionuclide content between similar size fractions in the presence and absence of 2 µM EDTA (*see* Fig. 3.4 *for details*). A. Net recycling of 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 particles >0.2 µm, grazing treatment, t = 3 h, n = 1).

treatments was estimated as the difference in radionuclide content of size fractions in the presence and absence of added EDTA (Fig. 3.7A). Since 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. 3.7A; controls) and the picoplankton size fraction (Fig. 3.7B; controls), this "desorbed" metal is assumed to be strongly bound by a ligand <5 kD (*see below*), possibly a low molecular weight organic complex excreted by the cyanobacterium. Since 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 approximately 20% greater than the net recycling of ^{65}Zn (16% of total radioactivity), as measured at the end of the experiment (Fig. 3.7A). At the end of the experiment, the majority of the recycled Cd was associated with the picoplankton (7.7%; Fig. 3.7B), followed by the nanoplankton (4.8%; Fig. 3.7C) and the microplankton (3.4%; Fig. 3.7D). In contrast, most of the recycled ^{65}Zn was associated with the nanoplankton (5.8%; Fig. 3.7C); the microplankton (1%; Fig. 3.7D) and picoplankton (≈0%; Fig. 3.7B) 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. 3.7A) and the sum of the recycled trace metal in each specific size fraction after 65 h (Fig. 3.7B-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 recycling; recycling represented only 11% of the ^{65}Zn accumulated by this plankton fraction after 65 h (Fig. 3.7D). 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% and 64% for ^{65}Zn and ^{109}Cd, respectively (Fig. 3.7C).

The molecular mass distributions of ^{109}Cd (77 ± 6% <5 kD) and ^{65}Zn (8 ± 24% <5 kD), as 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 (*cf.* Chapter Two). 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 kD was not significantly different from the dissolved (<0.2 μm) content in all the other treatments (prey control treatments ± EDTA, grazing treatment with EDTA), i.e. 100% ^{109}Cd and ^{65}Zn <5 kD. Radionuclides may be regenerated as various organic complexes which presumably reflect the localization of these metals in the prey item. The essential element Zn may be present as metallic co-factor in various enzymes such as the abundant enzyme carbonic anhydrase (molecular weight ≈30,000 a.m.u.), a contention supported by the size distribution of ^{65}Zn in the dissolved phase. Cadmium, a moderate substitute for Zn in carbonic anhydrase (Morel et al. 1994), may be co-excreted with this and other Zn-proteins, or be complexed as low molecular weight compounds such as free or combined amino acids, 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 review in* 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 with greater affinity for Zn and Cd than 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 using EDTA to trap regenerated 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; e.g. measured concentrations of dissolved (<0.2 μm) Zn and Cd at Stn. 84, 5 m, were 2.1 nM Zn and 39 pM Cd, 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. 3.4) suggest that EDTA was indeed complexing some of the regenerated ^{109}Cd and ^{65}Zn and thus, preventing its recycling.

The hypothesis of trace metal limited phytoplankton production in the surface waters of Lake Erie during thermal stratification is plausible. Low concentrations of dissolved trace metals in the surface water (Coale and Flegal 1989, Nriagu et al. 1993, Nriagu et al. 1996, this study–*see* Methods) coupled with moderate levels of phytoplankton biomass (≈1.7 μg chl-*a*·L^{-1}; Table 3.1), and the possible existence of strong complexing ligands of bioactive trace metals (*see above*), would all favour trace

metal limitation of phytoplankton. The three-fold greater trophic transfer efficiency of ^{65}Zn versus ^{109}Cd (Fig. 3.4) is consistent with a Zn-deficient plankton community.

Further evidence for a Zn-limited plankton community in this environment is provided by the dissolved Zn profiles from Lake Erie during summer months, as measured recently by Nriagu et al. (1996). These authors suggest that the severe depletion of Zn in surfaces waters is due to biological demand exceeding 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. 3.4) may have eliminated the need for organisms to utilize 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 will be required to establish the frequency and duration of trace metal limitation in pelagic Lake Erie plankton.

3.4.5 *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: i) microplankton grazers treat the radiolabeled *Synechococcus* as they do other members of the picoplankton community; ii) adding the radiolabeled spike does not radically alter the picoplankton community density; iii) 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 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 amounts of chl-*a* added to the experimental treatments as radiolabeled-*Synechococcus* were: 22%, 37%, and 18% (% total chl-*a*) for

the experiments at Stn. 23 (1994), Stn. 84 (1994), and Stn. 23 (1995), respectively. 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 addition of radiolabeled-*S. leopoliensis* represents a somewhat greater perturbation to ambient chl-*a* levels than to cell numbers.

A maximum autotrophic picoplankton density of 2.9×10^8 cells·L^{-1} in the eastern basin of Lake Erie during the summer has been observed (Weisse and Munawar 1989): the spikes of radiolabeled-*Synechococcus* used at Station 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·L^{-1} used in the experiment at Station 84 represented approximately 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}:$Zn_{0.012}$:$Cd_{0.005}$ (Stn. 23, 1994), C_{106}:$Zn_{0.004}$:$Cd_{0.0002}$ (Stn. 84, 1994), and C_{106}:$Zn_{0.002}$:$Cd_{0.0002}$ (Stn. 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}:$Zn_{0.034\text{-}0.1}$ (Reynolds and Hamilton-Taylor 1992). It is however possible 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 in the natural picoplankton community.

3.4.6 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, *cf.* Chapter Two, this study). With particular reference to trace metals, Hutchins et al. (1993) added radiolabeled-*Synechococcus* sp. (<5 μm) into water sampled from the equatorial and coastal Pacific Ocean, observed the appearance of radioactive Fe in organisms >5 μm, 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. In the experiments described in Chapter Two, radiolabeled-*Synechococcus* fed to the protozoan *Ochromonas* (a simplified food chain based on the microbial food web of the Laurentian Great Lakes) demonstrated 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 believe 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 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, Chapter Two and this chapter), 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. 1991a) 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, a re-examination of the functional role of microbial food web organisms in controlling trace metal geochemistry in surface waters is needed.

3.4.7 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, 1991a, 1991b), the reliable measurement of trace metal concentrations in these surface waters (Coale and Flegal 1989, Nriagu et al. 1993, Nriagu et al. 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 may at times 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 years (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.

3.5 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 to scavenging 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, is reasoned to 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, Nriagu et al. 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 counter-balance 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. 3.1B) 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).

CHAPTER FOUR

SCAVENGING OF ^{137}Cs, ^{109}Cd, ^{65}Zn, AND ^{153}Gd BY PLANKTON OF THE MICROBIAL FOOD WEB IN PELAGIC LAKE ERIE SURFACE WATERS

4.1 ABSTRACT

Scavenging–the sorption of particle-reactive trace metals to sedimenting particles–is generally accepted as the key factor controlling the concentration of dissolved trace metals in the surface waters of large lakes. Inorganic dissolved ^{137}Cs, ^{109}Cd, ^{65}Zn and ^{153}Gd were added, at concentrations well below their respective solubility limits, to water (<210 μm) sampled from the pelagic epilimnion of Lake Erie during the summers of 1994 and 1995. The hypotheses tested were that scavenging occurs in all of the ecologically significant size fractions that comprise the microbial food web (picoplankton, 0.2-2 μm; nanoplankton, 2-20 μm; microplankton, 20-210 μm), and that scavenging by plankton is directly related to the respective particle-reactivity (charge density; z^2/r) of the elements (Gd > Zn > Cd >> Cs). Size-selective filtration at intervals over 22-30 h established that picoplankton and nanoplankton were the dominant scavenging phases in this environment. Scavenging of ^{153}Gd, ^{65}Zn and ^{109}Cd by plankton was more similar than predicted on the basis of particle-reactivity; ^{137}Cs was weakly scavenged. Except for the picoplankton, ^{65}Zn was the element most readily scavenged by the plankton size fractions; high accumulation of ^{109}Cd in the picoplankton may reflect the sorption of this element by calcite associated with autotrophic picoplankton. These experiments provide new information on the partitioning of trace metals within the plankton of the microbial food web (0.2-210 μm), a dynamic community of particles that dominates the suspended particulate matter in the pelagic surface waters of Lake Erie during thermal stratification, and suggest that plankton dynamics should be considered in predictions of the geochemical fate of trace metals in this environment.

4.2 INTRODUCTION

Solid surfaces can scavenge particle-reactive metals by various sorptive mechanisms such as adsorption, complexation, precipitation, and internalization by organisms (Stumm et al. 1994). The gravitational loss of particles to the sediment controls the flux of trace metals in large lake systems, where the residence time of particles is much less than the hydraulic residence time.

The elemental composition of particles in the surface waters of the Laurentian Great Lakes shows strong seasonality. Analyses of the rare earth element content of particles retained in sediment

traps during isothermal conditions reveal that the majority of seston during this period is detrital material, whereas with the onset of thermal stratification epilimnetic particle composition shifts to autochthonous particles such as plankton and calcite (Robbins and Eadie 1991). Although calcite precipitation in the lower Great Lakes and Lake Michigan contributes a significant amount of particulate matter to the epilimnion during this time (Vanderploeg et al. 1987), the ability of calcite to scavenge most trace metals is considered to be inferior to that of biological surfaces (Sigg 1994), and even during whiting events particulate organic matter is generally considered to be the dominant phase responsible for the removal of particle-reactive trace metals from surface waters in large lakes (Robbins and Eadie 1991, Shafer and Armstrong 1991, Sigg 1994).

Particles in pelagic systems are dynamic. Earlier work on the scavenging of Cd and Zn by plankton considered that particles <28 μm in offshore surface waters of Lake Michigan were "mainly nonliving detrital material and mineral particles", especially during summer months (Parker et al. 1982). In their modeling of trace metal fluxes in Lake Michigan, Shafer and Armstrong (1991) considered biotic matter as sedimentable plankton, chiefly diatoms. The settling of this biological component of the seston undoubtably contributes to loss of trace metals from surface waters, but metal cycling events can occur prior to this event. In light of the subsequent progress made in understanding microbial food web dynamics in the Great Lakes (Fahnenstiel et al. 1986, Carrick and Fahnenstiel 1989, Fahnenstiel et al. 1991a, 1991b) and their influence on trace metal cycling (*cf.* Chapter Three), a re-examination of biotic influences on the geochemical fates of trace metals is warranted.

The microbial food web, wherein rapidly growing bacteria, cyanobacteria and algae in the picoplankton (0.2-2 μm) size class are intensely grazed by mixotrophic and heterotrophic consumer organisms in the nanoplankton (2-20 μm) and microplankton (20-200 μm) classes (Stockner and Porter 1988), is very active in surface waters of the Great Lakes (Weisse and Munawar 1989, Fahnenstiel et al. 1991a, 1991b). Given the ability of these consumer organisms, collectively referred to as microzooplankton, to regenerate C, N, and P due to the incomplete assimilation of these nutrients from their prey (Caron and Goldman 1990), it is conceivable that these microzooplankton will have comparable effects on trace metal cycling.

Indeed, rapid regeneration of ^{109}Cd and ^{65}Zn into the dissolved phase (<0.2 μm) was observed when radiolabeled picoplankton prey were grazed by the natural microzooplankton community (3-210 μm) present in the pelagic surface water of Lake Erie (*cf.* Chapter Three). In this latter study, marked differences in the trophic transfer and recycling of ^{109}Cd and ^{65}Zn from the radiolabeled prey into the

nanoplankton (3-20 μm) and microplankton (20-210 μm) were observed; the study demonstrated that the microbial food web has a significant influence on the partitioning of metals in surface waters, and suggested that the evolution of trace metal concentrations in surface waters during thermal stratification is linked to the intense growth and grazing activity within the <200 μm-planktonic community.

The objective of the present study was to examine the partitioning of trace metals from the dissolved phase into the plankton size fractions which comprise the microbial food web. Better appreciation of trace metal movement amongst various ecologically significant plankton size fractions should provide a basis for estimating how changes to the structure of the pelagic microbial food web, brought about by reduced nutrient loading or introduced species, will alter the geochemical fate of some trace metals. To this end, trace metal scavenging experiments were conducted in Lake Erie surface waters to evaluate the removal of cesium, cadmium, zinc, and the rare earth element gadolinium, chosen to represent a range in surface reactivity (Gd > Zn, Cd >> Cs). This study used an ecologically-based plankton size fractionation scheme (Sieburth et al. 1978) for classifying the seston into the picoplankton (0.2-2 μm), nanoplankton (2-20 μm) and microplankton (20-200 μm). Two hypothesis concerning the partitioning of these elements were tested: i) significant amounts of trace metal are accumulated within each of the ecologically significant size fractions, and ii) differences in relative affinities among the trace metals within a given plankton size fraction are attributable to differences in the particle-reactivity of the metals.

4.3 METHODS

4.3.1 Glassware/Plasticware Preparation

All plasticware and silanized glassware used in the experiments were rigorously cleaned prior to use. The cleaning protocol involved a warm soap wash (1% Liqui-Nox; Alconox, Inc.), methanol soak (HPLC grade; Baxter Healthcare Corp.), HNO_3 soak (1.6 M; Environmental Grade, Anachemia), and a soak in HCl (0.8 M; Suprapur, Merck), with a 7-fold rinse after each cleaning step using deionized water (≥17.5 Mohms·cm^{-1}).

4.3.2 Trace Metal Sorption Experiments

The sorption of the gamma-emitting radionuclides ^{137}Cs, ^{109}Cd, ^{65}Zn and ^{153}Gd by surface water plankton was studied at several pelagic stations in Lake Erie (Fig. 4.1) during the period of thermal stratification in the summers of 1994 and 1995. A profile of temperature and turbidity was

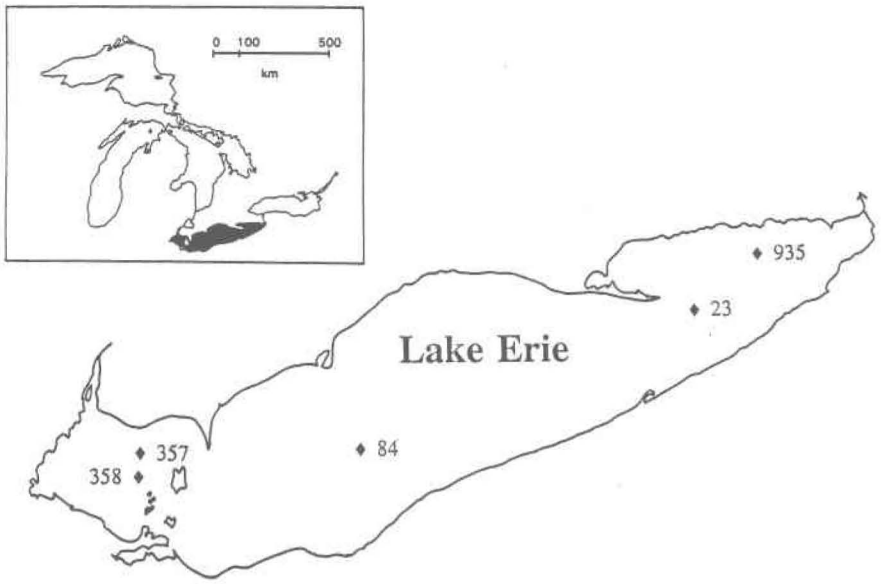

Figure 4.1. Study site locations in the pelagic zone of Lake Erie. Lake Erie metrics: volume = 499 km^3, surface area = 25,700 km^2, hydraulic residence time = 2.7 years (Quinn 1992).

obtained at each sampling station using a conductivity/temperature/depth (CTD) probe (Sea-Bird Electronics). Temperature and turbidity profiles at these stations revealed thermal stratification in the central and eastern basins and a well-mixed epilimnion at each of these stations, in contrast to the absence of stratification at the stations in the western basin (Fig. 4.2).

At each station, 16 L of lake water were collected from a depth of 5 m from the C.S.S. *Limnos* using an acid-cleaned, Teflon-coated 8-L Go-Flo bottle (General Oceanics) suspended on a non-metallic line. The sampling bottle was immediately transferred to a Class-100 portable cleanroom fixed to the deck of the ship where all subsequent manipulations were conducted. Lake water was filtered through a 210-µm pore size polypropylene mesh (Spectrum) and pooled, then 2 L was distributed to each of three 2-L polycarbonate bottles (Nalgene).

The scavenging experiments conducted during each of the three research cruises were relatively similar; the techniques employed in each cruise are described below. During the July 1994 cruise, a radioactive cocktail (75 µL) of ^{137}Cs, ^{109}Cd, ^{65}Zn and ^{153}Gd in 0.1 M HCl was added to each bottle immediately after the addition of an equivalent amount of NaOH (Gold Label, Johnson Matthey/Alfa-Aesar) to neutralize the acidity of the cocktail. Nominal added metal concentrations (and total radioactivities) were: Cs, 0.5 nM (133 kBq); Cd, 131 pM (111 kBq); Zn, 58 pM (122 kBq); Gd, 300 pM (56 kBq). Bottles were mixed gently by inversion, sampled for total radioactivity, and placed in an

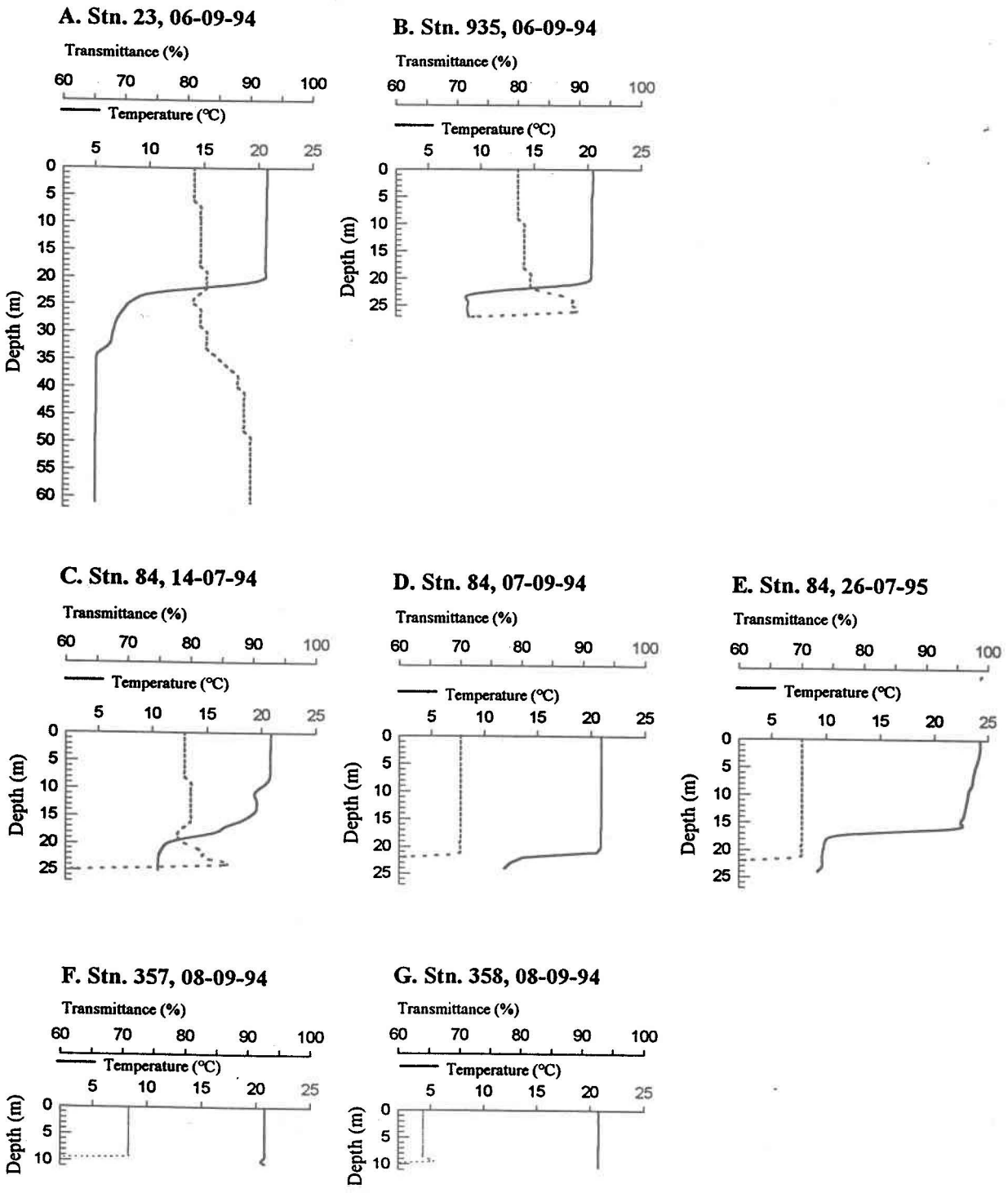

Figure 4.2. Water column temperature and turbidity profiles from stations in Lake Erie sampled for the study of trace metal scavenging by particles <210 µm. Station locations: Stns. 23 and 935, eastern basin; Stn. 84, central basin; Stn. 357 and 358, western basin.

incubator designed to simulate *in situ* conditions at 5 m (21°C and a light intensity of ≈120 μmol photons·m^{-2}·s^{-1}).

During the September 1994 cruise, the nominal added trace metal concentrations (and total radioactivities) were: Cs, 1 nM (266 kBq); Cd, 179 pM (139 kBq); Zn, 58 pM (122 kBq); Gd, 317 pM (42 kBq). All bottles were incubated on a shipdeck incubator, with temperature maintained at ambient surface water temperature (20°C) and natural light attenuated to 25% incident irradiance (e.g. 5 m depth) using 2 layers of neutral density screen. During the July 1995 cruise, the nominal added trace metal concentrations (and total radioactivities) were: Cd, 9.5 fM (33 kBq) and Zn, 44 pM (94 kBq); treatments during this cruise were incubated under simulated *in situ* conditions (20 ± 2°C, and 150 μmol photons·m^{-2}·s^{-1}).

At timed intervals over 22-30 h, the fate of the trace metal radionuclides was determined by serial size differential filtration. Partitioning of the radionuclides among the plankton size classes was determined by size fractionating 100-mL aliquots from the treatment bottles using a 20-μm screen (Nitex, 47 mm), followed by a 2-μm polycarbonate filter (Nuclepore, 47 mm), and finally, a 0.2-μm polycarbonate filter (Nuclepore, 47 mm). Volumes filtered at each fractionation were 100, 70 and 20 mL for the 20-, 2- and 0.2-μm filters, respectively. Applied vacuums were less than 13 kPa. The total volume of water removed from the sample bottles was less than 20%, except for the 1995 experiment (*see below*). At each step in the filtration series, filtrate was collected and removed, the filter was rinsed with 10 mL of filter-sterilized lake water (<0.2 μm), aspirated briefly to dryness, and removed. Two additional 2-mL samples from each bottle were removed at each sampling period to determine the total aqueous radioactivity.

During the July 1995 cruise size fractionation was conducted by parallel rather than serial filtration. Laboratory assays had established that there was no difference between results obtained for radionuclide partitioning if serial or parallel filtration was used to separate the delicate protozoan grazer, *Ochromonas danica*, from the picocyanobacterium *Synechococcus leopoliensis* (Appendix B). Parallel filtration was conducted for this single scavenging experiment since it allowed for numerous samples to be filtered in a short time period following the addition of the radioactive spike. In this method, separate aliquots of the sampled water were filtered directly onto a 20-μm filter, a 2-μm filter, or a 0.2-μm filter, and the filters were rinsed, as indicated above. Radionuclide accumulation by each plankton size fraction was determined by difference.

Water used in these scavenging experiments contained no mesozooplankton (>210 μm), which might have naturally influenced particle concentrations through grazing and defecation. In order to reduce the effect of this factor, the scavenging experiments were not run for more than ≈30 h. Based on the study of Parker et al. (1982), which showed that mesozooplankton (>253 μm) contribute only slightly to the total scavenging of radionuclides (≤3.5% ^{65}Zn, ≤7% ^{109}Cd), one would not expect the absence of particles >210 μm to alter greatly the partitioning of radionuclides in the present experiments.

4.3.3 Analytical Techniques

Radioactivity was determined with a gamma-spectrometer (LKB Wallac Compugamma 1282; NaI crystal) equipped with a multi-isotope assay option (UltroTerm software). The radioactivity of each radionuclide was measured in the following energy ranges: ^{109}Cd, 20-38 keV; ^{153}Gd, 125-189 keV; ^{137}Cs, 628-756 keV; and ^{65}Zn, 990-1,268 keV. Background radioactivity (lake water or filter blanks) was subtracted from the sample counts. Controls for measuring the amount of radionuclides adsorbed to filters were prepared by refiltering radiolabeled sterile lake water (<0.2 μm) through the appropriate filter and rinsing as usual. Propagated errors determined from the error of the sample and background counts were generally less than 10% (P<0.05).

At each station, one liter of the filtered (<210 μm) lake water was retained for measuring the initial pH and size-fractionated (0.2-2 μm, 2-20 μm, 20-210 μm) chlorophyll-*a* (chl-*a*) concentrations. Lake water pH was determined using the laboratory pH meter onboard the C.S.S. *Limnos*. Phaeophytin-corrected chl-*a* was determined onboard by fluorometric analysis after a 24 h 90% acetone extraction in the dark at 4°C (Parsons et al. 1984).

4.3.4 Grazing and Growth Assays

The dilution assay technique of Landry and Hassett (1982) was used to assess the grazing and growth rates of phytoplankton in the picoplankton, nanoplankton and microplankton size classes in water sampled from a depth of 5 m at Station 84 (July 1994) and Station 23 (September 1994). Details of the dilution assay procedure are outlined in Section 3.3.4.

4.3.5 Trace Metal Inorganic Speciation

Calculations of inorganic Cs(I), Cd(II), Zn(II), and Gd(III) trace metal speciation in Lake Erie surface water were conducted using a computerized chemical equilibrium model (MINEQL$^+$, version 2.23; Schecher and McAvoy 1991). Stability constants for Gd, Zn, Cd and Cs complexation were obtained from Turner et al. (1981). For the calculation of inorganic trace metal speciation, the ionic composition of Lake Erie water was based on a 1985 survey (Rockwell et al. 1989) of the central basin surface water during summer months (concentrations are mol·L^{-1}): Na^+, $3.8 \cdot 10^{-4}$; K^+, $3.4 \cdot 10^{-5}$; Ca^{2+}, $8.7 \cdot 10^{-4}$; Mg^{2+}, $3.4 \cdot 10^{-4}$; Cl^-, $4.1 \cdot 10^{-4}$; $Si(OH)_4$, $5 \cdot 10^{-6}$; PO_4^{-3}, $3.6 \cdot 10^{-8}$; NO_3^-, $1.4 \cdot 10^{-5}$; NH_3, $1 \cdot 10^{-6}$; SO_4^{-2}, $2.4 \cdot 10^{-4}$. Fluoride was considered to be $5.8 \cdot 10^{-6}$ mol·L^{-1} (Weiler and Chawla 1969). Chemical speciation for a system open to the atmosphere ($PCO_2 = 10^{-3.5}$ atm) was calculated for the pH range 6 to 9, at 22°C.

4.4 RESULTS AND DISCUSSION

4.4.1 Grazing and Growth Rates of Phytoplankton

Surface water sampled for scavenging experiments conducted at Stn. 84 (13-07-94) and Stn. 23 (06-09-94) was also assayed for chl-*a*-based specific rates of grazing and growth using dilution assays. Growth and grazing of autotrophic plankton occurred in all the surface water assayed (Fig. 4.3). In combination with dilution assays conducted at these stations on other dates and measurements of size-fractionated phytoplankton biomass (Table 4.1), these assays reveal a consistent trend of phytoplankton dynamics in the microbial food web of Lake Erie surface waters during summer (Table 4.2). The observed specific growth rates are within ranges published for Great Lakes photosynthetic picoplankton (0.05-1.5·d^{-1}; Fahnenstiel et al. 1986, 1991a, 1991b) and total phytoplankton growth (0.05-0.6·d^{-1}; Fahnenstiel and Scavia 1987, Scavia and Fahnenstiel 1987).

4.4.2 Trace Metal Sorption Experiments

For each of the added radionuclides, the majority of the metal did not sorb to plankton particles but remained in the dissolved phase. After 24 h, the average adsorptive losses to containers during assays were low: ^{153}Gd, 8.1 ± 5.0%, ^{65}Zn, 7.8 ± 3.9%; ^{109}Cd, 4.9 ± 3.7%; and ^{137}Cs, 2.5 ± 4.2% (measured as a decrease in total aqueous radioactivity). Nominal added concentrations of Zn (44-58 pM) were an order of magnitude less than average concentrations reported for pelagic Lake Erie surface

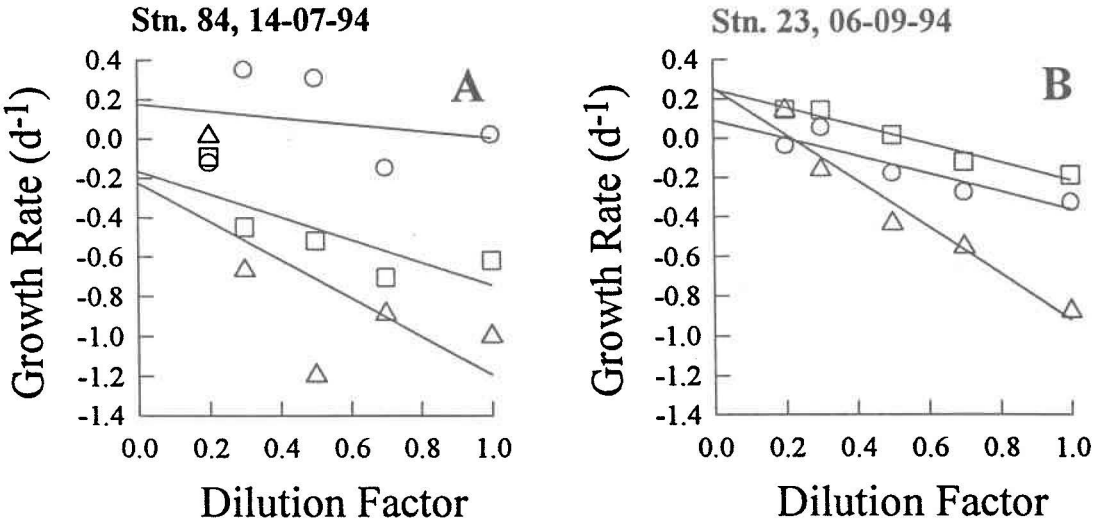

Figure 4.3. Results from dilution assays used to estimate the chlorophyll-*a* based rates of grazing and growth among the picoplankton (Δ), nanoplankton (□) and microplankton (○) size classes from the central basin (Fig. 4.3A) and eastern basin (Fig. 4.3B) of Lake Erie. Dilution factor is the ratio of whole lake water to sterile lake water in the treatment. The slope of the linear least squares regression of specific growth rate versus dilution represents the specific grazing rate whereas the intercept is the estimated intrinsic specific growth rate.

waters: 633 pM (Coale and Flegal 1989) and 1,330 pM (Nriagu et al. 1996). Added Cd (0.095-179 pM) was within, or below, the range of average reported Cd concentrations for this environment: 77 pM (Coale and Flegal 1989) and 249 pM (Nriagu et al. 1996). No reported measurements for dissolved stable cesium or dissolved gadolinium in Lake Erie could be found.

Metal added in the trace metal radionuclide spike to the lake water might not fully represent the ambient forms of dissolved metal in the sample. Equilibration of the radionuclides, added at concentrations well below their respective solubility limits, with inorganic dissolved ligands is expected to be established rapidly. However, isotopic equilibration with non-radioactive metal complexed by dissolved, or especially colloidal, organic matter is expected to be slower (Piro et al. 1972). Hence, the greater the proportion of non-labile metal present under ambient conditions, the

Table 4.1. Chlorophyll-*a* content of plankton size classes and characterization of water sampled from a depth of 5 m at various pelagic study sites in Lake Erie. Total chl-*a* does not include phytoplankton retained by a pre-filiter (210 µm-cutoff); water sampled from stations in the western basin had some algal/cyanobacterial filaments >210 µm. %Trans. = % transmittance at 750 nm.

Stn.	Date	Total chl-*a* ($\mu g \cdot L^{-1}$)	Chlorophyll-*a* (%) Micro	Nano	Pico	°C	%Trans.	pH
935[a]	06-09-94	2.26	26	34	41	20.4	80	8.2
23[b]	07-07-94	1.48	20	46	35	21.0	---	8.1
23[a,b]	06-09-94	1.91	19	32	49	20.8	81	---
23	24-07-95	1.88	31	38	31	22.0	---	8.5
84[b]	12-07-94	1.65	8	56	36	21.0	---	8.5
84[a]	13-07-94	1.81	9	57	34	20.8	79	8.5
84[b]	14-07-94	1.97	10	57	32	---	---	---
84[a]	07-09-94	4.57	13	43	44	21.0	70	---
84[a]	26-07-95	5.53	58	29	13	23.8	75	8.9
357[a]	08-09-94	2.58	47	29	24	20.8	71	---
358[a]	08-09-94	3.71	33	41	26	20.8	64	---

Notes: [a] Stations at which a trace metal scavenging experiment was conducted. [b] Stations at which grazing and growth rate assays of photosynthetic plankton were conducted.

Table 4.2. Chlorophyll-*a* based specific growth (µ) and grazing (g) rates (d^{-1} ± SE) in each plankton size fraction as determined using dilution assays. Calculated mean (± SD) is for the average of all four assays, with the exception of the negative growth rates determined for picoplankton and nanoplankton at Stn. 84 on 14-07-94 (*see* Fig. 4.3 *for details*).

Growth: Stn. and Date	Picoplankton	Nanoplankton	Microplankton
Stn. 84, 14-07-94	(-0.22 ± 0.40)	(0.17 ± 0.17)	0.17 ± 0.27
Stn. 84, 12-07-94[a]	0.50 ± 0.20	0.31 ± 0.08	0.68 ± 0.09
Stn. 23, 06-09-94	0.26 ± 0.10	0.25 ± 0.04	0.09 ± 0.07
Stn. 23, 11-07-94[a]	1.07 ± 0.10	0.74 ± 0.12	0.33 ± 0.08
Mean ± SD	0.61 ± 0.42	0.43 ± 0.26	0.32 ± 0.26
Grazing: Stn. and Date	Picoplankton	Nanoplankton	Microplankton
Stn. 84, 14-07-94	0.97 ± 0.63	0.57 ± 0.26	0.17 ± 0.41
Stn. 84, 12-07-94	1.60 ± 0.32	0.67 ± 0.12	0.18 ± 0.13
Stn. 23, 06-09-94	1.17 ± 0.16	0.46 ± 0.06	0.45 ± 0.11
Stn. 23, 11-07-94	1.93 ± 0.16	0.88 ± 0.19	0.28 ± 0.12
Mean ± SD	1.42 ± 0.43	0.65 ± 0.18	0.27 ± 0.13

Note: [a] values from dilution assays described in Chapter Three.

less representative would be the radionuclide addition, and the rate of scavenging obtained using the present experimental design would overestimate the true scavenging rate. Notwithstanding the question of isotopic equilibration, the addition of a largely labile pool of trace metal into the water samples, as conducted here, is similar to the atmospheric input of trace metals into these lakes (Lum et al. 1987); inputs of Zn and Cd are largely in soluble form, whereas no data are available for Cs or Gd solubility in wet or dry deposition).

Accumulation of trace metal radionuclides into all the plankton size fractions occurred rapidly after the addition of the radioactive cocktail (Fig. 4.4). At some stations partitioning among the aqueous and particulate phases was approaching an apparent sorption equilibrium within 20 h. Similar asymptotic sorption equilibria occurred within ≈24 h when carrier-free ^{109}Cd and ^{65}Zn were added to non-filtered water (containing mesozooplankton) sampled from the epilimnion of Lake Michigan (Parker et al. 1982).

The nanoplankton consistently accumulated the most radionuclides (eg. Fig. 4.4), except in the case of ^{109}Cd: this element was accumulated more in the picoplankton fraction than in the nanoplankton, with the notable exception of Stn. 84 (13-07-94; Fig. 4.4A'-A"). Although the picoplankton appeared to accumulate more ^{137}Cs than did the other size fractions after 20 h of exposure, this consistent trend was not statistically significant (P>0.05).

Sorption of trace metal radionuclides to particle surfaces is generally considered to be highly dependent upon the metal's reactivity (Fisher 1986), which is directly related to its first hydrolysis constant, or respective charge density (charge to ionic radius ratio; z^2/r): Gd^{3+} (9.6) > Zn^{2+} (5.4) > Cd^{2+} (4.1) > Cs^{+} (0.6). This range in reactivities was not however directly reflected by the accumulation of these radionuclides into the various plankton size fractions in the Lake Erie surface waters examined here. Cesium behaved as expected; the least surface reactive of the elements studied, it never accumulated into the sum of the plankton size classes to levels greater than 1.2% of the total ^{137}Cs added (with the exception of accumulation by seston from stations in the western basin; *see below*). However, the accumulations of Gd, Zn, and Cd by the plankton size fractions were more similar than expected.

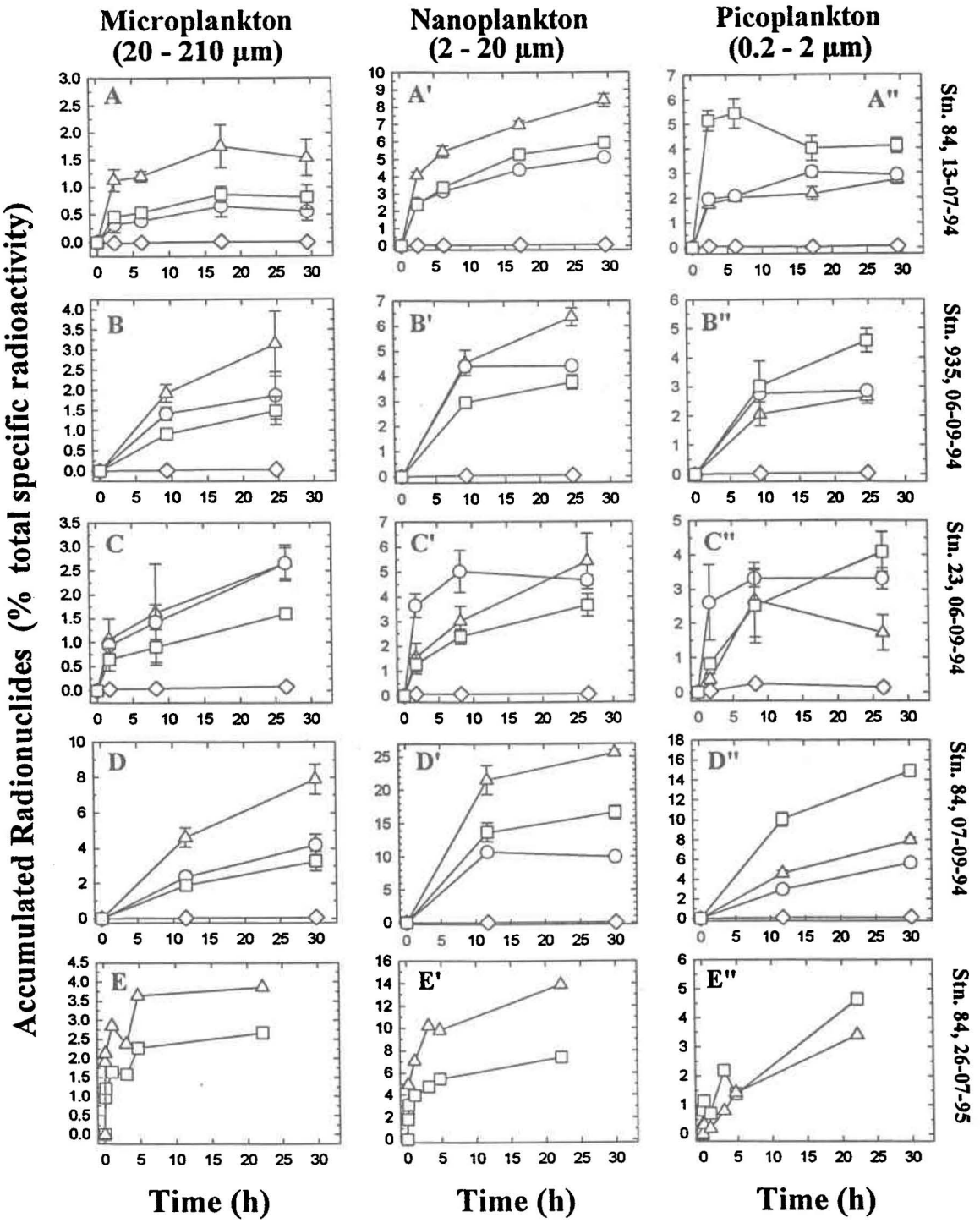

Figure 4.4. Sorption of trace metal radionuclides by various plankton size classes in surface water samples collected in pelagic regions of the central and eastern basins of Lake Erie. Study sites are arranged in an order of increasing total chlorophyll content (A < E; *see* Table 4.1). Values represent mean ± SD (n = 3), except E-E" where n = 1. Trace metals were added in an inorganic dissolved form to each treatment at the beginning of the exposure period. Total added metal concentrations were: Gd, 300-317 pM; Zn, 44-58 pM; Cd, 0.01-179 pM; Cs, 0.5-1 nM (*see* Section 4.3.2 *for details*): O – ^{153}Gd; Δ – ^{65}Zn; □ – ^{109}Cd; ◊ – ^{137}Cs.

Gadolinium – Despite the fact that ^{153}Gd is the most strongly hydrolysed element among the added radionuclides and was thus expected to be the most readily scavenged element, total ^{153}Gd accumulations by plankton were equal to, or less than, those of ^{65}Zn or ^{109}Cd (Fig. 4.4). Similar to these observations made in Lake Erie, the observed volume concentration factor of ^{153}Gd ($[^{153}Gd]_{algae}/[^{153}Gd]_{water}$) for the diatom *Skeletonema costatum* cultured in sea water (pH 8.2) was $10^{5.4}$, compared with a measured concentration factor of $10^{4.9\text{-}5.5}$ for ^{65}Zn (Bingler et al. 1989). The present results from Lake Erie, and these observed levels of ^{153}Gd sorption by a marine diatom, contrast with the relative accumulations of radionuclides by the picoplanktonic cyanobacterium *Synechococcus leopoliensis* in an inorganic growth medium (pH 7.5), similar in chemical composition to Lake Erie but containing no dissolved organic matter. In the laboratory assays, *S. leopoliensis* bioconcentrated ^{153}Gd to values $10^{6.4}$-times the aqueous concentration present in radiolabeled inorganic growth medium; this bioconcentration factor was approximately 10-fold greater than that for ^{65}Zn and ^{109}Cd. In preliminary studies of the present experimental design on surface waters of Lac Bedard, a circumneutral lake (pH 6.4, total chl-*a* = 6 $\mu g \cdot L^{-1}$) in the Laurentian Mountains north of Quebec City, total sorption of ^{153}Gd (9.3%) by particles after 25 h was far greater than that observed for ^{109}Cd (1.9%), ^{65}Zn (1.1%) and ^{137}Cs (0.3%). These differences in the accumulation of Gd may reflect differences between natural plankton and the cultured phytoplankton, those between natural waters and defined culture medium, or possibly the presence of a particulate mineral phase in Lac Bedard capable of preferentially binding Gd. However, variations in the ambient pH are likely the major factor affecting the sorption of Gd.

In support of the suggestion that Gd sorption is highly pH-dependent, inorganic speciation calculations indicate that Gd availability at the elevated pH of Lake Erie surface water in the summer was likely limited by the very low concentrations of inorganic labile Gd (e.g. the Gd^{3+} aquo-ion) for sorptive reactions (Fig. 4.5). At a total dissolved concentration of 300 pM Gd, the $[Gd^{3+}]$ would be only 0.06 pM at pH 9, but near 160 pM at pH 6.5. The presence of natural dissolved organic matter in surface waters may have also played a significant role in controlling Gd speciation; like all rare earth elements, Gd has a strong affinity for organic ligands (Sholkovitz 1995).

Cesium – Cesium speciation was dominated by the univalent Cs^+ cation (Fig. 4.5). Since over 99% of ^{137}Cs was in the dissolved phase in all sorption experiments (with the exception of the water sampled from the western basin; *see below*), the very low values for Cs sorption suggest that

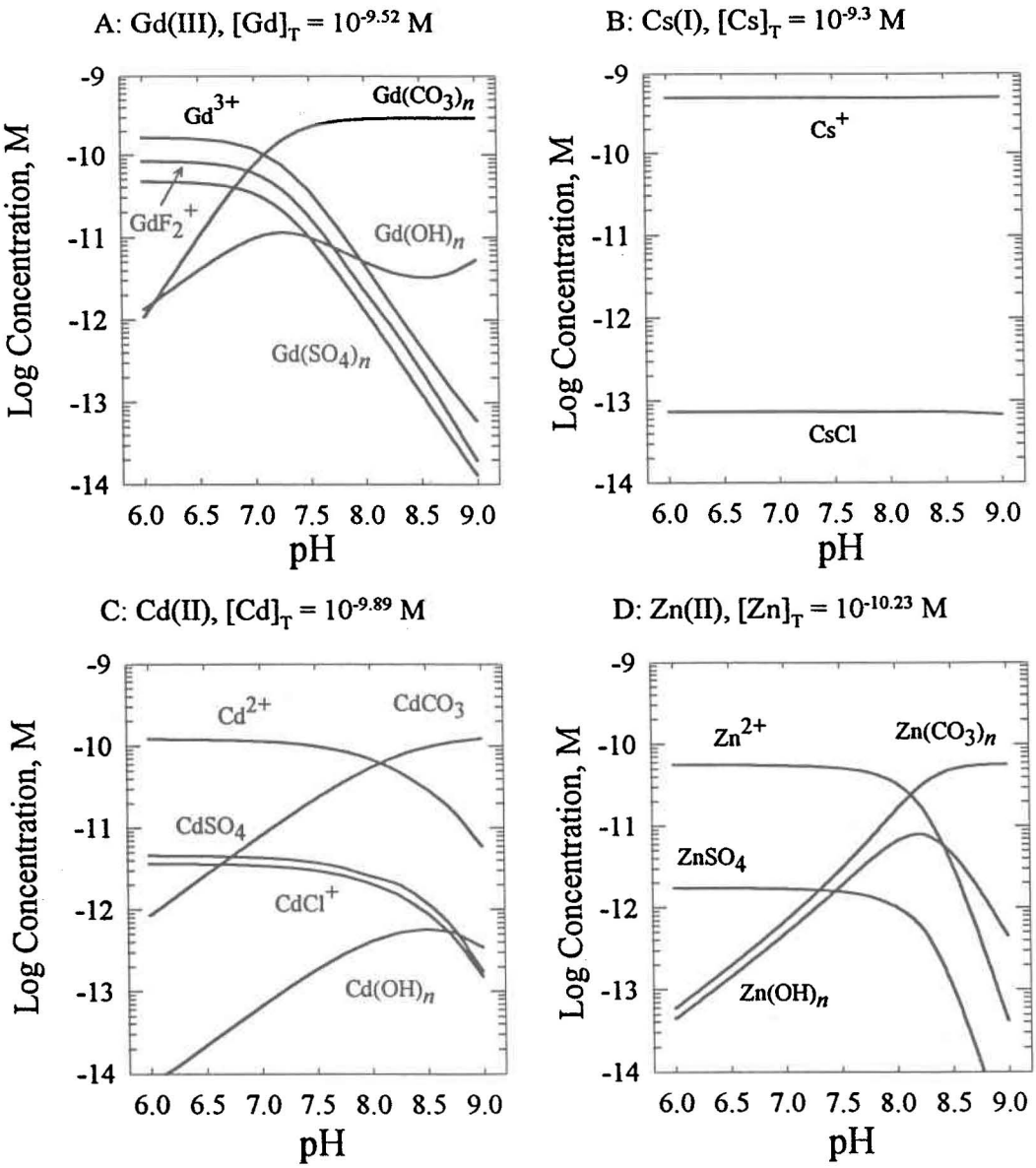

Figure 4.5. Inorganic speciation of trace metals studied in scavenging experiments in simulated Lake Erie surface water (summer, central basin) over the pH range 6-9 (*see* Section 4.3.5 *for a detailed description of water composition*). Note: *n* denotes the sum of trace metal bound to a specific ligand, e.g. $Gd(OH)n = GdOH^{2+} + Gd(OH)_2^{+} + Gd(OH)_3 + Gd(OH)_4^{-}$.

resuspended sediments (>0.2 μm) were not abundant in the pelagic surface waters. Cesium has a high affinity for sediments (Hesslein et al. 1980), especially inorganic detrital material such as clay (Francis and Brinklet 1976). The observed high level of total ^{137}Cs sorption to particles from the western basin (3.6% at Stn. 357, 4.8% at Stn. 358), where the surface water was contiguous with the sediment (Fig. 4.2), is consistent with a higher affinity of Cs^{+} for inorganic detrital materials than biological surfaces (Garnham et al. 1993).

Cadmium and Zinc – Differences in the relative amount of ^{109}Cd and ^{65}Zn accumulation by the nanoplankton and microplankton were consistently observed: significantly more ^{65}Zn than ^{109}Cd was accumulated into these two plankton fractions. The opposite was observed for accumulation in the picoplankton. It appears that Cd was favourably sorbed by the picoplankton, or conversely, that Zn sorption by this size class was inhibited. Given the abundant dissolved pool for both these elements in the sorption experiments (>85% of total), this difference in behaviour cannot be attributed to a lack of available metal for sorption by particles. Although the calculated proportion of the free metal ion at pH 8.5 was greater for Cd (23%) than for Zn (6%), chemical speciation cannot explain the consistent preferential sorption of Cd by the picoplankton–if so one would expect to find preferential Cd sorption on all particles, regardless of size.

One possible explanation for the differing behaviour of Cd and Zn lies in their different reactions with the surface of calcite. Laboratory studies have established that Cd is more readily sorbed onto the surface of calcite, and less easily desorbed, than is Zn (Zachara et al. 1991). The warm temperature (20+°C) and high pH (≥ 8.1) during the study period favoured the precipitation of biogenic calcite. It is conceivable that calcite formed on the surface of autotrophic picoplankton, as has been demonstrated for carbonate mineral formation on the surface of *Synechococcus* (Schultze-Lam and Beveridge 1994), and that this solid phase is responsible for the preferred sorption of Cd by the picoplankton.

4.4.3 Trace Metal Scavenging Potential of Various Plankton Size Fractions

Radionuclide accumulation into the various plankton size fractions was positively correlated to the chl-*a* content of a given size fraction (Fig. 4.6). Affinities of radionuclides for the various size fractions were derived from linear least squares regressions (Table 4.3). The affinity of ^{109}Cd and ^{65}Zn exceeded that of ^{153}Gd in all size fractions, and with the exception of the picoplankton, ^{65}Zn had a greater particle affinity than ^{109}Cd (Table 4.3). Note that estimated particle affinity (slope) is a composite value and presumably reflects both favourable binding sites on the particle surfaces, nutritive requirements (hence a high degree of internalization by microbiota in the case of Zn), as well as trophic transfer of radionuclides for size fractions containing consumer organisms (*cf.* Chapter Three).

Peak sorption of ^{109}Cd and ^{65}Zn by total seston in offshore waters of Lake Michigan (>0.45 μm) coincided with the period of maximum phytoplankton biomass in July (Parker et al. 1982); however, on

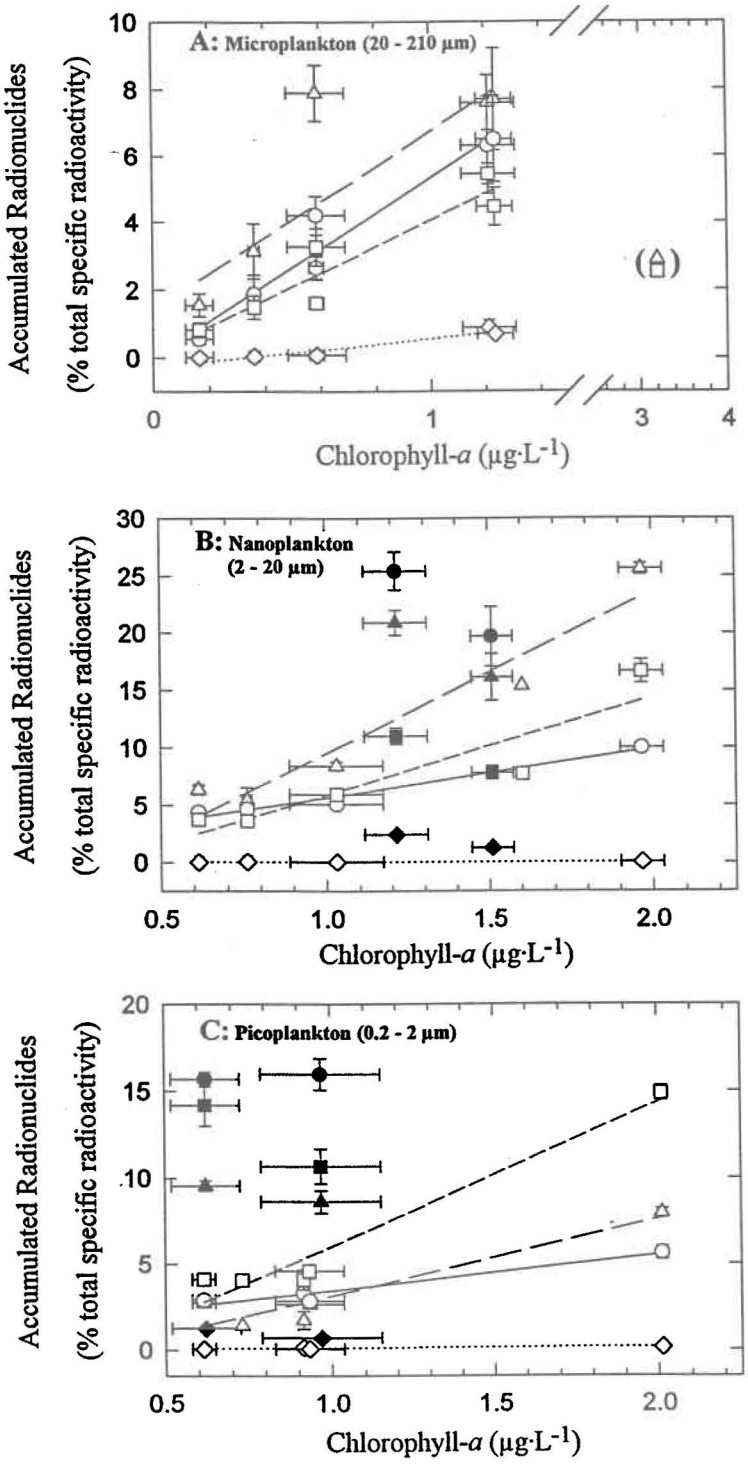

Figure 4.6. Chlorophyll-specific sorption of trace metal radionuclides by various plankton size classes in pelagic surface waters of Lake Erie. Plotted values represent the accumulated radionuclides (mean ± SD, n = 3) at the end of each assay (22-30 h) at the observed size-specific concentration of chlorophyll-a (mean ± SD, n = 2-3). Data for ^{109}Cd and ^{65}Zn sorption by microplankton at Stn. 84 (24-07-95) were not considered in the regression analyses; microplankton was dominated by a bloom of colonial *Microcystis* at this sampling time. Solid symbols representing data collected from the western basin were not included in the regression analyses but are included for comparative purposes (*see text for explanation*). Trace metal radionuclides: O – ^{153}Gd; Δ – ^{65}Zn; □ – ^{109}Cd; ◊ – ^{137}Cs.

Table 4.3. Linear least squares regression analyses of sorbed trace metal radionuclides over 22-30 h as a function of chlorophyll-*a* content in various plankton size classes. Regressions are plotted in Figure 6; linear formula is y = *m*x + *b*, where y = accumulated metal (% total specific radioactivity added), *m* = slope, x = concentration of chl-*a*, *b* = y-intercept. Values of *m* and *b* are expressed ± 95% confidence intervals. Regression correlation coefficients (r) are coded for significance levels: <1% = ****; <2% = ***; <5% = **; <10% = *; no coefficients are reported for *d.f.* <3.

Plankton Size	Metal	*m*	*b*	r	*d.f.*
Microplankton	^{153}Gd	5.33 ± 0.63	0.003 ± 0.62	0.973****	4
(210-20 μm)	^{65}Zn	5.32 ± 2.00	1.42 ± 1.97	0.800*	4
	^{109}Cd	3.94 ± 0.71	0.13 ± 0.70	0.940****	4
	^{137}Cs	0.83 ± 0.15	-0.27 ± 0.14	0.943****	4
Nanoplankton	^{153}Gd	4.29 ± 0.56	1.35 ± 0.59	0.984	2
(20-2 μm)	^{65}Zn	14.13 ± 2.31	-4.65 ± 2.65	0.962****	3
	^{109}Cd	8.54 ± 2.18	-2.66 ± 2.51	0.914**	3
	^{137}Cs	-0.001 ± 0.02	0.07 ± 0.02	0.041	2
Picoplankton	^{153}Gd	2.10 ± 0.36	1.33 ± 0.38	0.972	2
(2-0.2 μm)	^{65}Zn	4.50 ± 0.92	-1.38 ± 1.03	0.943***	3
	^{109}Cd	8.36 ± 1.06	-2.35 ± 1.19	0.977****	3
	^{137}Cs	0.08 ± 0.04	0.02 ± 0.05	0.781	2

an inter-seasonal basis (May to December), only the sorption of ^{65}Zn correlated significantly with primary productivity measured as $^{14}CO_2$ uptake. Since zinc is a required trace element, such a correlation between sorption and productivity is understandable. Based on the data from the present study, the hypothesized affinity of ^{109}Cd for biogenic calcite in the picoplankton size fraction (*see above*) may help to explain the significant correlation (P <0.01) between chl-*a* and the sorption of this element during the time period in which these scavenging experiments were conducted. Calcite precipitation is linked to primary productivity during periods of intense photosynthetic activity and warm water temperatures, normally from June to early September in Lake Erie. Thus, the link between Cd scavenging and primary production would only be evident when primary production induces calcite precipitation; this may explain the lack of a significant correlation between chl-*a* and Cd sorption in Lake Michigan over the period from May to December reported by Parker et al. (1982).

In the plots of radionuclide accumulation *versus* chl-*a* concentration, the estimated intercepts (Table 4.3) represent the average non-chl-*a*-specific sorption of a given radionuclide for a given size fraction. Only ^{153}Gd possessed significant non-chlorophyll-specific sorption in the nanoplankton and picoplankton fractions, whereas the regressions of ^{65}Zn and ^{109}Cd sorption by the nanoplankton and

picoplankton had negative intercepts. Further study in more oligotrophic environments would be required to explain these metal sorption/chl-*a* relationships fully.

Stations sampled in the western basin of Lake Erie were eliminated from the regressions for nanoplankton (Fig. 4.6B) and picoplankton (Fig. 4.6C) since resuspended sediment was probably affecting the composition of the seston. The epilimnion at these stations during the sampling period was contiguous with sediment whereas strong thermal stratification prevailed at all other stations, as indicated by the CTD profiles (Fig. 4.2). Wave action and shipping activity are suspected to have resuspended sediment at these sites in the western basin. The influence of non-chlorophyllous particles on the sorption of the radionuclides by the nanoplankton and picoplankton at these stations is evident in Figure 4.6. Since the resuspended sediment did not have a noticeable influence on radionuclide sorption in the microplankton size fraction (i.e. resuspended particles were presumably <20 μm), the values for accumulation by the microplankton at these stations were retained for the regression analyses.

The relationships between scavenged trace metal and chl-*a* plotted in Figure 4.6 assume that the amount of radionuclide scavenged by surfaces at each station was in the pseudo-linear section of a sigmoid curve (Fig. 4.7). The hypothesized sigmoidal relationship is similar to the titration of a constant concentration of metal with increasing amounts of ligand; this is a reasonable approximation of the assumptions made in these experiments where relatively constant concentrations of each metal were

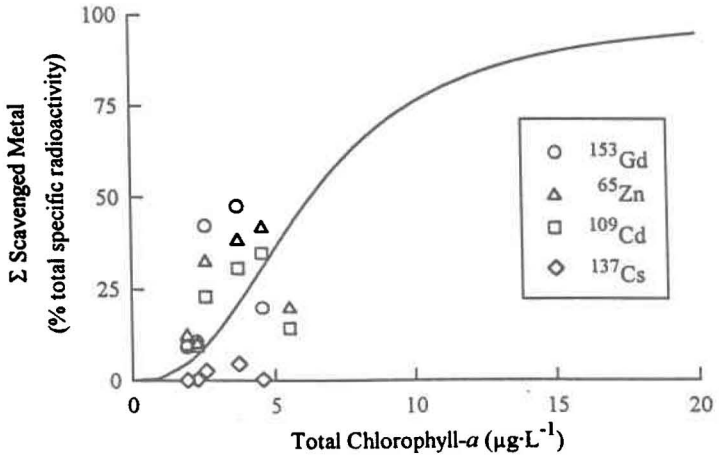

Figure 4.7. Total specific trace metal scavenging by the combined picoplankton, nanoplankton and microplankton versus the total concentration of chlorophyll-*a* measured at each study station. The solid curve represents an hypothesized sigmoidal relationship between specific total scavenged metal and chlorophyll-*a* for a system in which a constant total metal concentration is titrated with increasing quantities of particles with sorptive surfaces.

added to the water samples, and where chl-*a* was used as a surrogate measure of particle surfaces (ligands). The total concentration of chl-*a* decreases along a west to east transect; average summer epilimnetic values for the western, central and eastern basins are: 10.8, 3.2, and 1.4 $\mu g \cdot L^{-1}$ (Rockwell et al. 1989). In the case of the surface waters in the eastern and central basins, the total scavenging of trace metals is likely in the linear section of the hypothesized sigmoidal relationship (Fig. 4.7).

4.4.4 Biogeochemical Implications of Trace Metal Scavenging by Microbial Food Web Organisms

The relationship of trace metal sorbed per unit chl-*a* (Fig. 4.6) illustrates well the importance of the various ecologically significant size fractions to trace metal scavenging. However, such a relationship is mechanistically limited: it does not address the numerous means by which a metal can enter a given size fraction, namely, surface sorption, internalization (transmembrane uptake), trophic transfer, and the generation of particulate fecal matter resulting from consumption.

One possible method to control for the relative contribution of surface sorption would be to use a biological inhibitor. In this manner, metal accumulated by particles would be dominated by the surface sorption of metals. Such a technique was employed in preliminary experiments with surface water from Lac Bedard (*cf.* Appendix C) in which sodium azide (154 mM), formaldehyde (170 mM), or near freezing temperature (≈0°C) were used to inhibit biological activity. Radionuclide sorption into plankton fractions was compared with a treatment that received no inhibitor. In the presence of inhibitors, less ^{137}Cs, ^{109}Cd, ^{65}Zn and ^{153}Gd accumulated into the plankton in comparison with the living treatment (Appendix C). However, when metabolic inhibitors are used, interpretation of the observed scavenging behaviour is difficult since the value for partitioning in the controls (inhibitors present) refers to trace metal adsorbed to particles that existed when the inhibitor was added. Particle dynamics in a living sample (e.g. growth, consumption, fecal matter production) are such that the relative importance of different particle populations may well vary over time, generating subtle differences between living and inhibited treatments. Examples of microbial planktonic community dynamics are evident in the observed rates of phytoplankton growth and grazing in the plankton fractions <210 μm (Table 4.2). Thus, metabolic inhibitors have limited applicability in following the path of trace metals in a rapidly changing planktonic world.

In their study of Zn and Cd fate in Lake Michigan surface waters, Parker et al. (1982) observed that the majority of ^{65}Zn (≥63%) and ^{109}Cd (≥76%) scavenged by the seston >0.45 μm was scavenged

by the fraction 0.45-28 μm. A similar result was observed in the present study where the majority of scavenged trace metal (0.2-210 μm) after 20 h was scavenged by particles in the size fraction 0.2-20 μm (^{153}Gd, 83% ± 7%; ^{65}Zn, 80% ± 6%; ^{109}Cd, 85% ± 5%; ^{137}Cs, 76% ± 6%; mean ± SD). However, as documented in the present study, the subdivision of this wide particle size spectrum into ecologically meaningful size classes yields a better appreciation of the important geochemical roles that microbial food web organisms play.

The approach used here to assess the partitioning of trace metals within the plankton of the microbial food web (0.2-210 μm) has provided new information on trace metal fates in Lake Erie. The data support the hypothesis that significant amounts of trace metals are scavenged by ecologically important size fractions, primarily the picoplankton (0.2-2 μm) and nanoplankton (2-20 μm). The hypothesis that trace metal scavenging by plankton is proportional to the metal's particle-reactivity is partially supported by the data. For example Cs, the most weakly particle-reactive metal studied here, was also the most weakly scavenged. However, the chemical speciation of trace metals under the conditions present in Lake Erie surface waters plays an important role in limiting the availability of hydrolysable elements (e.g. Gd) for scavenging reactions. In addition, significant differences in the metal binding characteristics of particles among size fractions are considered to be the reason for the greater scavenging of Cd over Zn in the picoplankton size fraction.

Autochthonous particles such as plankton and calcite dominate the suspended particulate matter in pelagic surface waters during thermal stratification (Robbins and Eadie 1991), and the measurements of growth and grazing rates of pelagic Lake Erie phytoplankton <210 μm (Table 4.2) demonstrate that the autotrophic members of this autochthonous particle population are continuously changing. This suggests that plankton dynamics should be considered in predictions of the geochemical fate of trace metals in this environment. In Chapter 5, the trace metal and plankton dynamics reported here are incorporated into a dynamic model that is used to predict trace metal residence times and to evaluate the impact of the microbial food web on trace metal cycling in pelagic Lake Erie surface waters.

CHAPTER FIVE

TRACE METAL CYCLING IN THE SURFACE WATERS OF LAKE ERIE: LINKING ECOLOGICAL AND GEOCHEMICAL FATES

5.1 ABSTRACT

The loss of plankton from surface waters by sedimentation is considered to be the key factor controlling the concentration of trace metals in the surface waters of large lakes–the highly productive plankton that comprise the microbial food web are ideally suited to scavenging particle-reactive trace metals. The hypothesis that the microbial food web significantly influences the geochemical fate of trace metals was tested using a dynamic model of trace metal fate in the microbial food web. Observed characteristics of the various plankton size fractions in the microbial food web (ability to scavenge trace metals from the dissolved phase; potential to regenerate these metals back into the dissolved phase; population dynamics) were incorporated into the model. The model was used to estimate epilimnetic trace metal residence times (τ) under the assumption of steady state conditions (metal input = metal output): τ_{Cs} = 514 d, τ_{Cd} = 29 ± 32 d, τ_{Zn} = 32 ± 21 d, τ_{Gd} = 66 ± 43 d (mean ± SD). These metal residence times are significantly greater than the residence times predicted if microzooplankton grazing activity is eliminated from the model simulations (Cs, +46%; Cd, +62%; Zn, +58%; Gd, +84%). The prolongation of residence time by microzooplankton grazing is attributed to the regeneration of trace metal that results from the incomplete assimilation by the grazer of metal previously scavenged by the prey item. A simulated increase in the dominance of picoplankton biomass in pelagic Lake Erie (15-70% total phytoplankton biomass) increased residence times of Zn (+17%) and Gd (+22%), but not Cd (-24%). The results illustrate the important influence of the microbial food web on the geochemical fates of trace metals in the pelagic surface waters of large lakes during thermal stratification.

5.2 INTRODUCTION

The microbial food web is a significant component of the trophic structure in the Laurentian Great Lakes (Caron et al. 1985, Pick and Caron 1987, Carrick and Fahnenstiel 1989, Weisse and Munawar 1989, Fahnenstiel et al. 1991a, Fahnenstiel et al. 1991b). Since the grazing activity in this food web serves to regenerate macronutrients such as phosphorus and nitrogen (Caron and Goldman 1990) and divert organic carbon from higher trophic levels in the water column (Azam et al. 1983, Ducklow 1983), it seems logical to consider the implicit importance of trace metal scavenging by

picoplankton and the hypothesis of trace metal regeneration from the picoplankton by microzooplankton grazing.

The importance of the planktonic scavenging phase (≤28 μm) has been demonstrated for the scavenging of ^{109}Cd and ^{65}Zn in water sampled from the offshore of Lake Michigan (Parker et al. 1982). In a series of scavenging experiments, described in Chapter Four, the majority of the ^{137}Cs, ^{109}Cd, ^{65}Zn and ^{153}Gd scavenged from the dissolved phase in water sampled from pelagic Lake Erie surface waters was attributed to plankton ≤20 μm. Note, however, that two competing phenomena exist with respect to trace metal-plankton interactions and their impact on the fate of dissolved trace metal in surface waters: losses due to scavenging of the original metal inputs, and recycling resulting from the scavenging of regenerated metal. The grazing activity of microzooplankton might be expected to regenerate metal scavenged by their prey back into the dissolved phase where scavenging can re-occur. Indeed, each cycling event can alter the partitioning of the trace metals within the water column, as was demonstrated in a laboratory model of a simplified microbial food web (Chapter Two) and verified in pelagic Lake Erie surface waters (Chapter Three). Thus, microzooplankton grazing activity might serve to increase trace metal residence times both chemically, through the production of organic colloids which would bind trace metals in solution and reduce their availability for subsequent sorption, and physically, by the production of metal-bearing colloidal fecal matter having a low sedimentary loss rate.

The objective of this study was to incorporate the microbial food web into existing models of trace metal cycling based on steady-state principles (Table 5.1); this allowed predictions of trace metal movement amongst various plankton size fractions and provided a basis for estimating how changes to the structure of the pelagic microbial food web could alter the geochemical fate of some trace metals. The study was restricted to the pelagic surface waters of Lake Erie during thermal stratification; during this period, both the lack of contact between epilimnion and sediment and the peak in microbial food web activity will tend to accentuate the influence of the microbial food web on trace metal cycling.

The hypothesis that microzooplankton grazing can regenerate trace metals has been confirmed in both laboratory (Chapter Two) and field (Chapter Three) studies. The corollary of this hypothesis is that trace metal regeneration will increase the residence times of these elements in these systems. The objective of this modeling effort is to quantify the effect of trace metal regeneration by the microbial food web significantly on the residence time of particle-reactive trace metals in pelagic surface waters. A second objective is to determine to what extent an increase in the proportion of picoplankton relative to the entire plankton community in Lake Erie will increase trace metal residence times. A significant effect on residence times is considered here to be an increase or decrease of 10%.

Table 5.1. Models based on steady state principles for estimating trace metal residence times (τ_M).

Model		Characteristics
I.	Mass Balance	–estimates τ_M using measured [M] in surface waters and an estimated rate of metal input based on atmospheric loading; data obtained from published values of loading and [M].
II.	Static Plankton	–estimates τ_M by calculating the rate of metal loss due to scavenging by plankton; the estimate is dependent upon plankton growth, biomass, and scavenging potential; data obtained primarily from literature sources; grazing not included.
III.	Dynamic Plankton	–estimates τ_M by calculating the rate of metal loss due to scavenging minus regeneration due to grazing. Model uses measured rates of plankton grazing, scavenging potential, biomass, metal regeneration, and metal fractionation in the dissolved phase determined from field and laboratory studies, in addition to published values for the rate of metal input.

5.3 METHODS

5.3.1 Trace Metal Residence Times in an Open System under Steady State Conditions

The three modeling approaches used here to estimate trace metal residence times in Lake Erie surface water (Table 5.1) are all constructed on the principle of steady state, where constant metal inputs from fluvial and atmospheric sources into the system (the well-mixed epilimnion) equal sedimentary losses to the hypolimnion and sediment (Fig. 5.1). The residence time of a trace metal in the epilimnion is determined by estimating the loss rate of total metal from the system:

$$\tau_M = [M_T]/(\delta[M_T]/\delta t) \qquad (5.01)$$

where τ_M = residence time, $[M_T]$ = total concentration of trace metal in the epilimnion, and $\delta[M_T]/\delta t$ = the net rate of metal movement through the system.

All values are for residence times (τ_M) reported here have been corrected for the hydraulic residence time of Lake Erie (τ_W = 986 d): $\tau_M^{-1} = \tau_W^{-1} + \tau_S^{-1}$, where τ_S = the residence time for trace metal with respect to loss by sedimentation (as predicted directly by the steady state models).

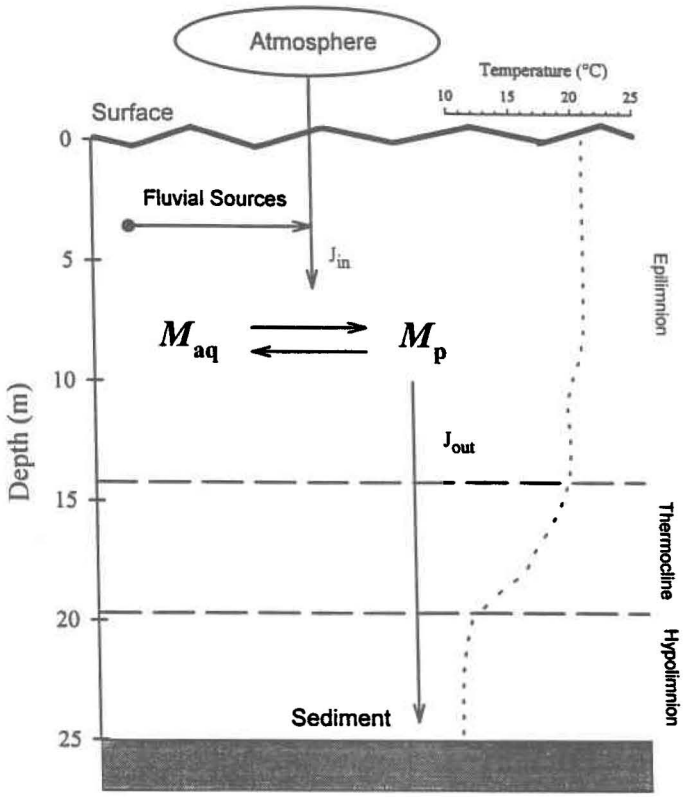

Figure 5.1. Schematic diagram of particle-reactive trace metal flux in a thermally stratified pelagic water column. Metal flux (J_{in}) into the epilimnetic surface water is equal to the flux of metal leaving the epilimnion (J_{out}). Sedimentary loss of particles through the thermocline is the primary loss vector of particle-reactive trace metals from surface waters. The thermocline is an effective density-gradient barrier; during thermal stratification the resuspension of sediment into the epilimnion is prevented.

5.3.2 Mass Balance Model for Predicting Trace Metal Residence Time

The mass balance model is based on reported dissolved metal concentrations during thermal stratification and estimated trace metal fluxes into pelagic Lake Erie. Since the system is at steady state, the input rate equals the rate of loss from the system, which is assumed to be the sedimentary loss of particulate matter. At present, the only available data on the trace metals studied here are for Cd and Zn (Coale and Flegal 1989, Nriagu et al. 1996). The residence time was estimating using Equation 5.01, the Zn and Cd concentrations listed in Table 1.1, and Cd and Zn fluxes reported by Nriagu et al. (1996).

The input fluxes for the trace metals are based on measurements reported in the literature or estimations. Zinc and cadmium fluxes into Lake Erie are 6.07 and 0.26 $pmol \cdot L^{-1} \cdot d^{-1}$, respectively. These input rates are based on reported yearly atmospheric fluxes of 3.33×10^{-5} mol $Zn \cdot m^{-2}$ and 1.42×10^{-6} mol $Cd \cdot m^{-2}$ (Nriagu et al. 1996), and an estimated epilimnion volume of 3.86×10^{14} L (lake surface area = 2.57×10^{10} m^2 [Quinn 1992], epilimnion depth = 15 m).

5.3.3 A Static Plankton Model of Trace Metal Fates in Surface Water

The following model assumes that autochthonously produced particles are the primary scavenging phase in pelagic epilimnetic waters during the summer. Once metal is scavenged it is assumed to leave the system by instantaneous and complete sedimentation. The rate of scavenging that controls metal residence time (Equation 5.01) is estimated from the production of biomass in a plankton size fraction, and from the ability of this biomass to sorb metals:

$$\delta[M_T]/\delta t = ß \times VCF \times [M_T] \qquad (5.02)$$

where ß = rate constant of biomass production (d^{-1}), VCF = volume concentration factor ($[M]_{biomass}/[M]_T$; $mol \cdot L^{-1}/mol \cdot L^{-1}$).

Plankton Biovolumes and Surface Areas – Trace metal scavenging by each plankton fraction was estimated using Equation 5.02. The picoplankton were separated into heterotrophic and autotrophic sets. Heterotrophic picoplankton cell volumes were based on spring and summer cell volumes of bacteria from surface waters of pelagic Lake Michigan (Scavia et al. 1986). The cell volume of autotrophic picoplankton was estimated from electronic particle size analysis of a phycoerythrin-rich *Synechococcus* sp. isolated from Lake Huron (gift from G.L. Fahnenstiel); samples of this species were fixed with Lugol's Iodine (1.5%) and analyzed using a Coulter Multisizer II; 15 µm orifice. Cell numbers of heterotrophic picoplankton (Wiesse and Munawar 1989), and autotrophic picoplankton (Wiesse and Munawar 1989, Pick 1991) were based on previously published measurements from the central and eastern basins of Lake Erie during summer months.

Average cell volumes of the members of the nanoplankton and microplankton were estimated from the extensive phytoplankton survey of Lake Erie conducted by Makarewicz (1993); however, this survey did not include autotrophic picoplankton biomass. The species-based biomass measurements of phytoplankton from the central basin of Lake Erie during spring and summer from 1983-1987 (Makarewicz 1993) were re-classified into plankton of the nanoplankton and microplankton size fractions based on the metric descriptions of the species (Prescott 1951, Tiffany and Britton 1952). Mean abundance-weighted cell volumes were then determined to be 54 $\mu m^3 \cdot cell^{-1}$ (n = 26) for the nanoplankton, and 961 $\mu m^3 \cdot cell^{-1}$ (n = 18) for the microplankton. Cell dimensions were solved from these mean cell volumes and the predominant cell morphology (surface area estimates assumed that cell surfaces were smooth, which might underestimate the true surface area of some species that have

relatively rough outer cell surfaces). The species listed by Makarewicz (1993) represent 89% of the phytoplankton biovolume measured in his survey.

Plankton Growth Rates – Growth rates of the autotrophic picoplankton, nanoplankton and microplankton were based on the results of dilution assays conducted during three research cruises (mid-July 1994, July 1995 and early September 1994; Table 4.2). These chl-*a*-based estimates are within the range of plankton growth rates measured by other researchers in the Laurentian Great Lakes during the summer (*see* Section 4.4.1). The growth rates of the heterotrophic bacteria were based on the measurements made by Scavia et al. (1986) on surface water sampled from Lake Michigan during spring and summer; growth rates ranged from 1.2 to $5.8 \cdot d^{-1}$.

Estimating the Scavenging of Trace Metals by Plankton – The volume concentration factor (VCF) for phytoplankton accumulation of a trace metal from the dissolved phase was assumed to be proportional to the surface area:volume ratio (A:V) of the organism (Appendix D), as shown for some trace metals with several unicellular marine phytoplankton (Fisher 1985). The estimated relationships of VCF to A:V for freshwater phytoplankton were as follows: VCF_{Cs} = 80.2 (A:V) + 271; VCF_{Cd} = 7,200 (A:V) + 14,600; VCF_{Zn} = 8,440 (A:V) + 32,100. In the case of Gd, which has a comparatively high reactivity with particle surfaces, the absence of adequate Gd bioaccumulation data as a function of A:V for freshwater or marine phytoplankton restricted any similar approximation. The static plankton model was run using an arbitrary VCF_{Gd} value of $2.5 \cdot 10^5$ for all plankton fractions, which is 10-fold less than VCF_{Gd} obtained with *Synechococcus* ($10^{6.4}$; obtained at pH 7.5, *cf.* Chapter Two). However, as discussed in Section 4.4.2, the VCF for Gd is expected to be lower at the higher pH found in Lake Erie surface waters (pH 8-9).

5.3.4 A Dynamic Plankton Model of Trace Metal Fates in Surface Water

Equation 5.01 is an adequate representation of a steady-state system for use in static models. However, the application of a dynamic model that allows for the return of scavenged metal to the aqueous phase requires further description.

The open system steady-state model depicted in Figure 5.2 is the basic model used in the dynamic model developed here. Under the condition of steady state, the rate of metal entering the system, v_{in}, equals the rate at which metal leaves the system, v_{out}, and accordingly, the concentration

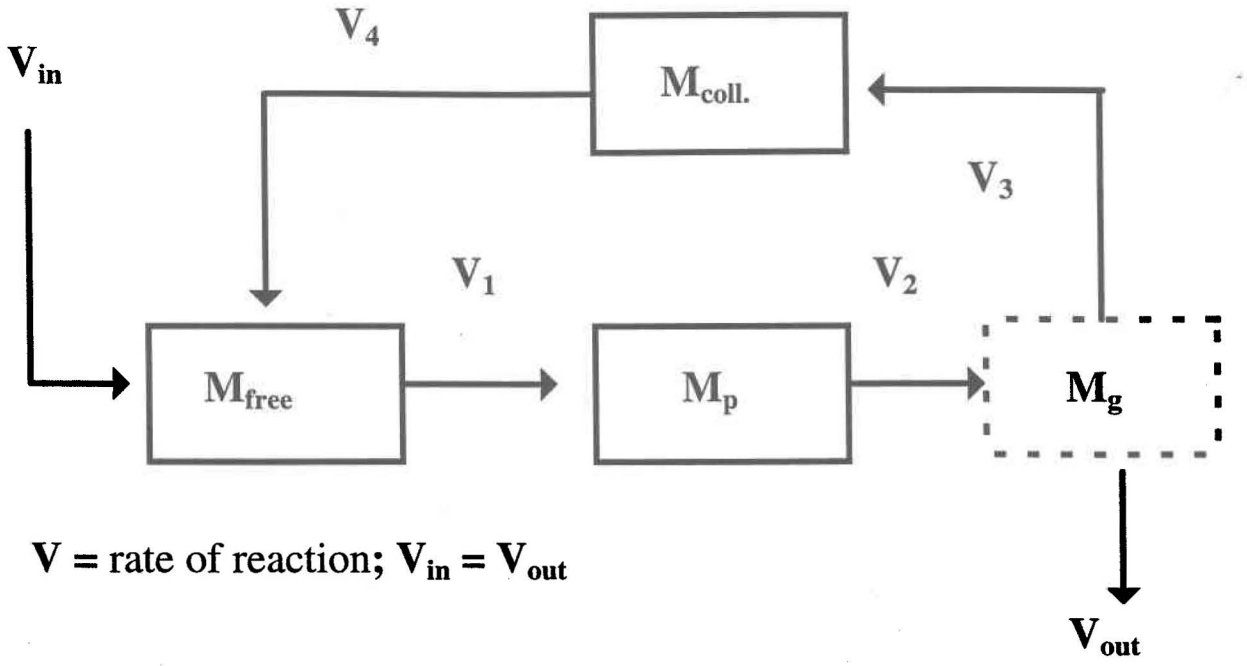

Figure 5.2. A dynamic plankton scavenging model of trace metal fate in the pelagic surface waters of Lake Erie during thermal stratification, based on steady-state principles. v_{in} is the rate of trace metal input into the system; v_1 represents rate of trace metal scavenging by plankton; v_2 is the rate at which scavenged metal (M_p) is grazed; v_3 is the rate at which grazed metal (M_g) is regenerated into the colloidal fraction of trace metal in the dissolved phase ($M_{coll.}$); v_4 is the rate at which regenerated trace metal enters the non-colloidal fraction of metal in the dissolved phase (M_{free}); v_{out} is rate at which trace metal, remaining after incomplete assimilation following grazing, leaves the system. v = velocity of trace metal movement. $v = k[M]$; where: $v = mol \cdot L^{-1} \cdot d^{-1}$, $k = d^{-1}$, $[M] = mol \cdot L^{-1}$.

of metal in each compartment, labile metal (M_{free}), colloidal metal ($M_{coll.}$), and particulate metal (M_p), remains constant. The equations used to describe the movement of metal among the various compartments are described in Table 5.2. Rate constants and state variables used in the model are listed in Table 5.3.

As in the static plankton model, the dynamic plankton model is based on the scavenging of trace metal by the production of biomass. The ecological fate of the plankton then controls the geological fate of the trace metal–either trophic transfer through grazing activity, which ultimately leads to sedimentation, or regeneration back into the dissolved phase for recycling once more through the microbial food web. The dynamic model assumes steady-state plankton dynamics (grazing rate = growth rate) during the simulation period. Chl-*a* is a surrogate measure of plankton biomass in this model. As a first approximation, the population dynamics of the heterotrophic plankton are assumed to

be identical to those of the autotrophic plankton in the same plankton size fraction. Although bacteria have higher intrinsic growth rates than autotrophic picoplankton, the scavenging experiments make no distinction between the autotrophic and heterotrophic members of any size class.

Table 5.2. Differential equations used to describe plankton dynamics and metal partitioning within the dynamic plankton model depicted in Figure 5.2. The model is an open system at steady state with respect to metal flux and plankton biomass.

$$\delta M_p/\delta t = k_1[M_{free}] - k_2[M_p] \quad (5.04)$$

$$\delta M_g/\delta t = k_2[M_p] - k_3[M_g] - v_{out} \quad (5.05)$$

$$\delta M_{coll.}/\delta t = k_3[M_g] - k_4[M_{coll.}] \quad (5.06)$$

$$\delta M_{free}/\delta t = k_4[M_{coll.}] + v_{in} - k_1[M_{free}] \quad (5.07)$$

Where:

$k_1 = (s_{micro}\cdot\mu_{micro}\cdot b_{micro}) + (s_{nano}\cdot\mu_{nano}\cdot b_{nano}) + (s_{pico}\cdot\mu_{pico}\cdot b_{pico})$

s = scavenging potential; $(\mu g\ chl\text{-}a\cdot L^{-1})^{-1}$

μ = specific growth rate; d^{-1}

b = biomass; $\mu g\ chl\text{-}a\cdot L^{-1}$

$k_2 = g_{micro} + g_{nano} + g_{pico}$; d^{-1}

$k_3 = fM_{regen.}$; d^{-1}

$k_4 = fM_{free}$; d^{-1}

Note: $\mu = g$, under steady state conditions; k = reaction rate constant (d^{-1}); $[M]$ = concentration of metal ($mol\cdot L^{-1}$).

Particulate Phase, M_p – The rate constant for scavenging of labile metal, k_1, is a composite value based on each plankton size fraction (Table 5.2). The scavenging of trace metal by particles is weighted for the specific growth rate, biomass, and scavenging potential of each plankton size class (Table 5.3). Biomass production ($chl\text{-}a\cdot L^{-1}\cdot d^{-1}$), the product of the biomass (b) and growth rate (μ) is assumed to scavenge trace metal from the M_{free} compartment in accordance with the scavenging potential specific for each size fraction (s; $\%M\cdot(\mu g\ chl\text{-}a^{-1}\cdot L^{-1})^{-1}$). Thus, when the scavenging potential is expressed as a fraction for use in the model calculations, biomass production is converted into scavenged metal, M_p. In turn, M_p is subject to loss due to grazing, at the same specific rate under which it is formed (since $\mu = g$, under steady-state conditions).

Table 5.3. State variables and parameters used to describe the behaviour of plankton biomass and the partitioning of trace metals among the dissolved and planktonic phases in the dynamic plankton model.

		Plankton Size Fractions		
		Microplankton (20-210 μm)	**Nanoplankton** (2-20 μm)	**Picoplankton** (0.2-2 μm)
State Variable				
Biomass[a], μg chl-*a*·L^{-1}:		0.54 ± 0.41	1.13 ± 0.28	0.90 ± 0.26
Parameters[b]				
Specific growth and grazing rates, d^{-1}:		0.27 ± 0.13	0.65 ± 0.18	1.42 ± 0.43
Scavenging[c], %·(μg chl-*a*·L^{-1})$^{-1}$	Gd:	5.3 ± 0.6	4.3 ± 0.6	2.1 ± 0.4
	Zn:	5.3 ± 2.0	14.1 ± 2.3	4.5 ± 0.9
	Cd:	3.9 ± 0.7	8.5 ± 2.2	8.4 ± 1.1
	Cs:	0.8 ± 0.2	-0.001 ± 0.02	0.08 ± 0.04

	Trace Metal			
	Gd	**Zn**	**Cd**	**Cs**
$fM_{regen.}$[d], d^{-1}	0.88	0.59	0.62	0.98
fM_{free} [e], d^{-1}	0.31 (0.74)	0.08	0.77	1.0

Notes: [a] Values are mean ± SD (n = 9), determined from data collected at stations in the eastern and central basins only (*from* Table 4.1). [b] Specific growth and grazing rates are chl-*a* based rates, mean ± SD, n = 4 (*from* Table 4.2). [c]Values for the scavenging water are the slope (± 95% confidence intervals) of % total radionuclide scavenged per μg chl-*a*·L^{-1} (*from* Table 4.3). The scavenging potential of nanoplankton for Cs was considered to be 0.001 ± 0.02 in the model simulations. [d]Regeneration was calculated as the fraction of the consumed trace metal that was released into the aqueous phase by grazing activity (values for Cd and Zn from field experiments [Chapter Three]; values for Cs and Gd from laboratory experiments [Chapter Two]). [e]The fraction of trace metal regenerated by microzooplankton grazing that is <5 kD and considered to be labile, fM_{free}, was derived from field and laboratory experiments.

Grazed Particulate Metal, M_g – Within a given time interval the entire mass of metal that enters the grazed metal compartment leaves; therefore, M_g is not considered to be among the pools of accumulated metal, as are M_p, $M_{coll.}$, and M_{free}. Grazed metal is either regenerated into the aqueous phase, or leaves the system by sedimentation, v_{out} (Equation 5.05). Grazing is assumed to remove trace metal at a constant rate, k_2 = specific grazing rate (Table 5.3), regardless of the plankton fraction responsible for the grazing, or the plankton fraction being grazed. However, the specific rate at which metal is regenerated from grazed biomass, $k_3 = fM_{regen.}$ (Table 5.3), is metal-specific. Regenerated metal was estimated from the proportion of metal that entered the aqueous phase per unit of metal consumed as picoplanktonic prey, as determined in field and laboratory experiments: values for the percent Cd and Zn regeneration were obtained from field experiments in Lake Erie (Cd = 62%, Zn = 59%), whereas regeneration of Cs and Gd was determined in laboratory experiments (Cs ≈ 98%, Gd = 88%). For the purpose of the modeling, these proportions regenerated are expressed per unit of time (d^{-1}).

The sedimentary loss of residual metal that remains in the M_g compartment after grazing ($v_{out} = k_2[M_p] - k_3[M_g]$) assumes that the loss of plankton biomass as zooplankton fecal pellets is rapid relative to the residence time of the trace metal in the system. This model does not consider the gravitational loss of aggregated colloids from the system. Since the average residence time (τ) of particles leaving the shallow epilimnion of Lake Erie (≈ 15-20 m) is considered to be much shorter than the residence time of colloids in fresh water, then v_{out} can be assumed to be dominated by the sedimentary loss of particulate metal, i.e.:

$$1/\tau_{Mp+coll} = 1/\tau_{Mp} + 1/\tau_{Mcoll.} \qquad (5.08)$$

For example, if τ_{Mp} = 7 d and $\tau_{Mcoll.}$ = 60 d, then $\tau_{Mp+coll} \approx 6.3$ d.

Colloidal Metal in the Aqueous Phase, $M_{coll.}$ – Regenerated trace metal is considered to consist of both colloidal, $M_{coll.}$ and free metal, M_{free}. The measured size fractionation of regenerated trace metal radionuclides in the dissolved phase following micrograzing was used as the parameter to set the rate, k_4, at which free trace metal is excreted by grazers. The fractionation of trace metal between colloidal and non-colloidal metal is based on the extent to which radionuclides regenerated into the dissolved phase by microzooplankton grazing activity passed through a 5 kD molecular weight cutoff ultrafilter. For the purpose of the modeling, this fractionation is assumed to take place instantaneously and is expressed per unit of time.

The size distributions of ^{109}Cd (77 ± 6% <5 kD) and ^{65}Zn (8 ± 24% <5 kD) observed in the dissolved phase of natural Lake Erie surface water to which radioactive picoplankton were added

(Chapter Three) are comparable to those measured in the laboratory: ^{109}Cd, 73 ± 18% <5 kD; ^{65}Zn, 25 ± 21% <5 kD (Chapter Two). The only measurement made for the fractionation of ^{153}Gd resulting from microzooplankton regeneration is a single measurement of 74% <5 kD (Chapter Two). In contrast, Sholkovitz (1995) reports that 69% of the Gd in Connecticut River water is >5 kD, which suggests that the majority of dissolved Gd in that aqueous environment is bound by colloids. Cesium was considered to be entirely present as non-colloidal metal (Chapter Two). The values for fM_{free} were thus: 1, 0.77, and 0.08, for Cs, Cd, and Zn, respectively; the response of the model to two fM_{free} values (0.74 and 0.31) for Gd was assessed (Table 5.3).

Non-colloidal Metal in the Dissolved Phase, M_{free} – Only free metal, M_{free} is considered to be available for scavenging reactions. In essence, the dynamic model serves to simulate an atmospheric input event wherein a significant fraction of the trace metal atmospheric input arrives in soluble or dissolved form (Lum et al. 1987) and mixes into the epilimnion. Of course, in the real world some portion of this dissolved form is equally available for complexation reactions with colloids, thus yielding a $M_{coll.}$ fraction. The model does not deny this possibility. However, since colloidal metal is assumed to be relatively stable in the water column (i.e. no appreciable sinking rate) it will not significantly affect the rate at which metal leaves the dissolved phase (*cf.* Equation 5.08). Hence, the model traces the fate of bioavailable metal, from the sorption by particles to incorporation into particulate matter, and regeneration into the $M_{coll.}$ compartment.

The rate of input the trace metals in the dynamic plankton model was based on measurements reported in the literature or estimations. Cadmium and zinc inputs into Lake Erie are 0.26 and 6.07 $pmol \cdot L^{-1} \cdot d^{-1}$, respectively (*see* Section 5.3.1). The input rates of Cs (0.093 $pmol \cdot L^{-1} \cdot d^{-1}$) and Gd (0.21 $pmol \cdot L^{-1} \cdot d^{-1}$) were based on estimations of 100 pM Cs and 10 pM Gd in the surface water of Lake Erie; the model was used to solve for the input rates required to achieve these estimated total metal concentrations at steady state.

Estimating Trace Metal Residence Time – Trace metal partitioning among the dissolved and particulate phases over time was simulated in the dynamic plankton model using Stella II software (Version 3.0.7; High Performance Systems Inc., Hanover, NH). Numerical solutions of differential equations in the dynamic plankton model were simulated using Euler's method.

The loss rate of trace metal from the entire system is inversely proportional to the residence time of trace metal in the system. In the dynamic model (Fig. 5.2), trace metal residence time was

estimated by dividing the sum of metal in each compartment ($[M_T] = [M_{free}] + [M_{coll.}] + [M_p]$) by the net rate of metal moving through the system, since $v_{in} = v_1 - v_3 = v_{out}$ at steady state:

$$\tau_M = [M_T]/(v_1 - v_3) \qquad (5.09)$$

The residence time of trace metal in the system in the absence of any grazer-mediated regeneration (i.e. eliminating v_3) can be estimated by dividing $[M_T]$ by v_1, the gross rate of metal scavenging by the plankton.

5.3.5 Sensitivity Analysis

The sensitivity of the static plankton model to the variability of certain parameters (forcing functions, e.g. plankton growth rates) was calculated using the following formula:

$$S = |\delta x/x| \div |\delta P/P| \qquad (5.09)$$

where S is the sensitivity index of parameter, P, to variations in the state variable, x (Jorgensen 1994).

Stella® software allows for simulations which incorporate a "sensitivity analysis" of all parameters and state variables simultaneously. In this case, the specific value for a parameter or state variable was randomly chosen by the software within a range defined by the normal distribution of its estimate. When the variability of all parameters and state variables is incorporated into the model, an indication of the entire model's precision in estimating τ_M was obtained.

5.4 RESULTS AND DISCUSSION

5.4.1 The Static Plankton Model of Trace Metal Scavenging by Various Plankton Size Fractions

A simple estimation of biotic particle control of trace metal residence times in surface water is provided by the static plankton model. The model (Table 5.4) predicts that the concentration of trace metal associated with biomass is relatively small in comparison with the concentration of dissolved metal: Cs, 0.05%; Cd, 4%; Zn, 6%; Gd, 14% (metal in biomass/dissolved metal x 100). Such a response is expected in an environment where the total concentration of particles is small (although particles possess a much greater concentration of trace metal than the surrounding environment, they represent a small fraction of the total metal present in the epilimnion).

As expected on the basis of their high A:V ratio and their high growth rates (greater than the other plankton classes), the combined heterotrophic and autotrophic picoplankton scavenge a disproportionately large amount of trace metal relative to their biomass. The static plankton model

indicates that the majority of all trace metals would be scavenged by the picoplankton size fraction (70% Cs, 75% Cd, 69% Zn, 43% Gd), despite the low standing crop of trace metal present in this size fraction (32% Cs, 38% Cd, 31% Zn, 13% Gd). Thus, with respect to biomass, the scavenging by the picoplankton size class represents at least a four-fold greater amount of scavenging over the nanoplankton and microplankton size classes (Table 5.4). However, since the picoplankton do not have an appreciable sinking rate, the model implicitly assigns the loss of this scavenged biomass to grazing activity which keeps picoplankton biomass levels low and leads to the eventual transfer of scavenged trace metal to larger, more settleable particles (e.g. zooplankton feces).

Sensitivity Analysis: Static Plankton Model – The heterotrophic picoplankton size fraction was the most sensitive compartment in the static plankton model as revealed in a sensitivity analysis of the model output for each plankton fraction. The parameters of growth rate or VCF value were varied by 25% for each individual size fraction, and the sensitivity was calculated as the change in τ_M. Sensitivities were: Cs = 0.56, 0.04, 0.16, 0.23; Cd = 0.78, 0.04, 0.10, 0.07; Zn = 0.65, 0.04, 0.14, 0.17; Gd = 0.40, 0.03, 0.21, 0.36, for the heterotrophic picoplankton, autotrophic picoplankton, nanoplankton, and microplankton, respectively (note that in the absence of empirical data the VCF_{Gd} was held constant among all size fractions in the model; Table 5.4E).

Of all the plankton fractions, the heterotrophic picoplankton growth estimate was based on the widest range in values (the observed range of bacterial growth rates in pelagic Lake Michigan surface waters during spring and summer is 1.2-5.8$\cdot d^{-1}$; Scavia et al. 1986). If we assume similar growth behaviour of planktonic bacteria in Lake Erie and hold all other plankton fraction growth rates constant, then these growth rates are reflected in an estimated range in τ_{Cs} of 760-510 d, τ_{Cd} of 41-13 d, τ_{Zn} of 28-10 d, and τ_{Gd} of 8-4 d.

Table 5.4. The calculation of the trace residence time in pelagic Lake Erie surface waters (central basin) during summer based on the sorptive loss of metal from the dissolved phase to planktonic biomass. H = heterotrophic, P = photosynthetic.

A. Plankton Production

	Plankton Size Class:				
Parameters	**Hpico**	**Ppico**	**Pnano**	**Pmico**	
Shape:	sphere	sphere	sphere	box	
diameter	0.5	0.8	2.35	31.0	μm
width				5.57	μm
height				5.57	μm
Area (A)	0.79	2.01	17.4	753	μm^2
Volume (V)	0.066	0.27	6.80	962	μm^3
A:V	11.97	7.50	2.55	0.78	
Specific Growth Rate	2.0	0.61	0.43	0.32	d^{-1}
Standing Crop:					
Cell Number	$1.71 \cdot 10^9$	$1.12 \cdot 10^8$			$cells \cdot L^{-1}$
Biomass	$1.13 \cdot 10^8$	$3.00 \cdot 10^7$	$2.82 \cdot 10^8$	$6.43 \cdot 10^8$	$\mu m^3 \cdot L^{-1}$
Production:[a]					
Biomass	$2.26 \cdot 10^8$	$1.83 \cdot 10^7$	$1.21 \cdot 10^8$	$2.06 \cdot 10^8$	$\mu m^3 \cdot L^{-1} \cdot d^{-1}$

B. Rate of Cesium Scavenging: where, $[Cs]^b = 100$ pM

	Hpico	**Ppico**	**Pnano**	**Pmico**	
VCF	1,230	873	476	332	
Biomass[c]	0.014	0.003	0.013	0.021	$pmol \cdot L^{-1}$
Scavenging[d]	0.028	0.002	0.006	0.007	$pmol \cdot L^{-1} \cdot d^{-1}$

	Combined Pico	**Nano**	**Micro**	
Proportion of Biomass Cs	32	26	42	%
Proportion of Total Scavenging	70	14	16	%
Scavenging:Biomass	2.2	0.5	0.4	

Σ of Plankton Fractions: $\tau_{Cs} = 2{,}380$ days[e]; corrected for τ_W, $\tau_{Cs} = 700$ d.

Table 5.4. (continued)

C. Rate of Cadmium Scavenging: where, $[Cd]^f = 97$ pM

	Hpico	Ppico	Pnano	Pmico	
VCF	$1.01 \cdot 10^5$	$6.86 \cdot 10^4$	$3.30 \cdot 10^4$	$2.02 \cdot 10^4$	
Biomass	1.1	0.2	0.9	1.3	$pmol \cdot L^{-1}$
Scavenging	2.2	0.1	0.4	0.4	$pmol \cdot L^{-1} \cdot d^{-1}$

	Combined Pico	Nano	Micro	
Proportion of Biomass Cd	38	26	36	%
Proportion of Total Scavenging	75	12	13	%
Scavenging:Biomass	2.0	0.5	0.4	

Σ of Plankton Fractions: $\tau_{Cd} = 31$ days; corrected for τ_W, $\tau_{Cd} = 30$ d.

D. Rate of Zinc Scavenging: where, $[Zn]^g = 584$ pM

	Hpico	Ppico	Pnano	Pmico	
VCF	$1.33 \cdot 10^5$	$9.54 \cdot 10^4$	$5.37 \cdot 10^4$	$3.87 \cdot 10^4$	
Biomass	8.8	1.7	8.8	14.5	$pmol \cdot L^{-1}$
Scavenging	17.5	1.0	3.8	4.7	$pmol \cdot L^{-1} \cdot d^{-1}$

	Combined Pico	Nano	Micro	
Proportion of Biomass Zn	31	26	43	%
Proportion of Total Scavenging	69	14	17	%
Scavenging:Biomass	2.2	0.5	0.4	

Σ of Plankton Fractions: $\tau_{Zn} = 22$ days; corrected for τ_W, $\tau_{Zn} = 22$ d.

E. Rate of Gadolinium Scavenging: where, $[Gd]^h = 10$ pM

	Hpico	Ppico	Pnano	Pmico	
VCF	$2.51 \cdot 10^5$	$2.51 \cdot 10^5$	$2.51 \cdot 10^5$	$2.51 \cdot 10^5$	
Biomass	0.3	0.1	0.7	1.6	pM Gd
Scavenging	0.6	0.05	0.3	0.5	pM $Gd \cdot d^{-1}$

	Combined Pico	Nano	Micro	
Proportion of Biomass Gd	13	26	60	%
Proportion of Total Scavenging	43	21	36	%
Scavenging:Biomass	3.3	0.8	0.6	

Σ of Plankton Fractions: $\tau_{Gd} = 7$ days; corrected for τ_W, $\tau_{Gd} = 7$ d.

← **Notes to Table 5.4:** [a] Production of biomass = standing crop biomass x growth rate. [b]Estimated concentration of stable Cs. [c]*M* contained in biomass = ([*M*] x VCF x Standing Crop Biomass)/10^{15} $\mu m^3 \cdot L^{-1}$. [d]Scavenging = ([*M*] x VCF x Production of Biomass)/10^{15} $\mu m^3 \cdot L^{-1}$. [e]Turnover time (τ_M) = [*M*]/(δ[*M*]/δt), where δ[*M*]/δt = loss rate (scavenging), and [*M*] = total dissolved zinc. [f]Total dissolved Cd: Lake Erie, Stn. 84 (surface; August, 1987), Coale and Flegal 1989. [g]Total dissolved Zn: Lake Erie, Stn. 84, August 1987; Coale and Flegal 1989. [h]Estimated from a La:Gd ratio of 15:1, value for Lake Champlain (E.R. Sholkovitz, pers. comm.).

Static Plankton Model: Critique – In the static plankton model, trace metal scavenged by biomass is assumed ultimately to leave the surface water by sedimentation. However, this model does not account for the ecological fate of the plankton, nor for the fate of its associated trace metal, in the events preceding sedimentation. Since the grazing activity evident in the microbial plankton from the surface waters of Lake Erie rapidly and efficiently regenerates particulate-bound ^{109}Cd and ^{65}Zn into the dissolved phase (Chapter Three), some attempt must be made to address this phenomenon (see below).

5.4.2 The Dynamic Plankton Model of Trace Metal Turnover in the Microbial Food Web: Linking Geochemical and Ecological Fates

The series of scavenging experiments described in Chapter Four illustrates well the importance of the various ecologically significant size fractions in trace metal scavenging; these relationships are expressed as percentages of total trace metal sorbed per unit of chl-*a*, a surrogate for particle surfaces in pelagic surface waters. However, such a relationship is mechanistically limited. It does not address the numerous means by which a metal can enter a given size fraction, namely, surface sorption, internalization (transmembrane uptake), trophic transfer, and the generation of particulate fecal matter resulting from consumption.

The inability to account adequately for the dynamics of particles is viewed as one drawback to the use of one-box steady state models that incorporate surface complexation principles to predict trace metal residence times in surface waters (Schindler 1989). The dynamic plankton model presented here incorporates both trace metal and biological (particle) behaviour in surface waters. The purpose of this approach is first to establish if biological productivity can explain the observed dissolved metal concentrations in pelagic Lake Erie surface waters, and secondly, to determine if our current understanding of the influences of the microbial food web on trace metal fates can be used to explain how changes to the planktonic community may alter trace metal residence times in these waters. The model assumes that the biological activity of the plankton at a depth 5 m is representative of the entire mixed surface layer, or epilimnion.

At steady state, the dynamic plankton model predicted that the major fraction of the total metal in the surface water would be present in the dissolved phase. Particulate metal would represent a small

fraction of the total metal (Gd, 3%; Zn, 5%; Cd, 6%; Cs, ≤0.1%; Table 5.5) as expected in a system with a low particle load and as predicted by the static plankton model (Section 5.4.1). The proportion of Zn present as particulate metal is essentially the same between the two plankton models. However, the dynamic plankton model predicted a much smaller fraction of Gd as particulate material (3%) than did the static plankton model (14%). The discrepancy between scavenging potentials derived from the field (pH 8.1-8.5; presence of dissolved organic matter) and those estimated from the laboratory (pH 7.3; very low dissolved organic matter) may be the reason for this difference. In the case of Cd, the dynamic model predicted approximately twice the amount in the particulate fraction (6%) as compared to the static model (3%); the greater accumulation of Cd predicted by the dynamic model is attributed to the high affinity of Cd for plankton fractions observed under field conditions (especially the picoplankton).

5.4.3 Estimating the Influence of Microzooplankton on Trace Metal Residence Times

The behaviour of trace metals in the microbial food web was artificially manipulated in the dynamic plankton model by suppressing the production of regenerated trace metals into the aqueous phase. In all cases the presence of trace metal regeneration by grazing activity in the microbial food web dramatically increased τ_M with respect to the τ_M estimated in the absence of grazing (Table 5.4). *These results support the hypothesis that microzooplankton grazing activity will significantly increase (>10%) trace metal residence times in surface waters.*

A long residence time for Cs was consistently predicted by both the static and dynamic plankton models (Table 5.4B, Table 5.5). Since the τ_{Cs} predicted by the dynamic model is much greater than the hydraulic residence time of Lake Erie, it follows that the primary loss for Cs is via the outflow. The following analysis of the effect of microzooplankton grazing and community structure will thus concentrate only on the more particle-reactive elements: Gd, Zn and Cd.

Sensitivity Analysis of the Dynamic Plankton Model – The size fractionated chl-*a* measurements in the central and eastern basins of Lake Erie over a 2 month span reported in Table 4.2 suggest that the relative plankton abundance in the pelagic surface waters is reasonably stable over this time period. The predicted trace metal residence times of ≤60 d for Cd and Zn (Table 5.5) imply that the assumption of steady-state plankton dynamics within the residence time of the trace metals is likely a valid approximation during the mid to late summer season.

Table 5.5. Trace metal residence times and the partitioning of trace metal in the surface water of Lake Erie calculated using the dynamic plankton model under conditions of steady state plankton biomass. The model was simulated for Gd with fM_{free} = 0.31; if fM_{free} = 0.74, then Gd_{free} = 9.4 pM, $Gd_{coll.}$ = 0.3 pM, and Gd_p = 0.3 pM.

		Partitioning of Metal (pM)			Residence Time (d) Regeneration:		
Trace Metal	Rate of Loss ($pmol \cdot L^{-1} \cdot d^{-1}$)	M_{free}	$M_{coll.}$	M_p	No	Yes	Increase
Gadolinium	0.21	9.4	0.6	0.3	26	47	84%
Zinc	6.07	94	45	7.1	15	24	58%
Cadmium	0.26	4.5	0.2	0.3	12	19	62%
Cesium	0.09	99.8	0.09	0.13	350	514	46%

The global sensitivity of the model was assessed by estimating τ_M over numerous simulations of the model in which the state variables (biomass) and selected parameters (growth rate, grazing rate, scavenging potential) were randomly selected from within a defined range (Table 5.3). The variability in the parameters and state variables was established from the field measurements, and was simulated in the model using a Monte Carlo approach to randomly select a value within the range defined by the 95% confidence intervals about the mean. In the present case, our interest lies in the estimation of trace metal residence times. The precision of the model was not high when the inherent variability was incorporated into the estimates (Table 5.6). The coefficients of variation (SD/mean x 100) for the model simulations were: Gd, 39%; Zn, 36%; Cd, 53%.

Since the dynamic plankton model used measured values for the rate of Cd and Zn entry into Lake Erie, then the range in τ_M estimated by the sensitivity analysis (Table 5.6) can be used to predict a range in ambient concentrations of these elements in the surface water, i.e. $\tau_M \times v_{in} = [M]$. The range in concentrations (3-60 pM Cd, 100-890 pM Zn) predicted by the dynamic plankton model is comparable to the range of concentrations reported for the pelagic surface water of Lake Erie during thermally stratified conditions (13 ± 7 pM Cd, 370 ± 50 pM Zn; Nriagu et al. 1996). Concentrations of 630 ± 190 pM Zn reported by Coale and Flegal (1989) fall within the range predicted by the model, whereas their values for Cd (77 ± 14 pM) lie well above the range of modeled Cd concentrations. The scavenging potentials used in the dynamic plankton model may overestimate actual *in situ* scavenging and thus underestimate average ambient dissolved trace metal concentrations. The scavenging potentials are based on the addition of inorganic forms of the trace metals (*cf.* Chapter Four); in the case of Zn and Gd, the input of these elements may be in a chemical form that is considerably less available for

scavenging reactions. Any overestimation of trace metal scavenging by the plankton will lead to an underestimate of the ambient dissolved trace metal concentration at steady state.

Table 5.6. Sensitivity analysis of trace metal residence times calculated using the dynamic plankton model with steady state plankton biomass. Values τ_M (days), mean ± standard deviation (SD) for τ_M were calculated from 100 model simulations per trace metal in which parameters (growth rate, grazing rate, scavenging potential) and variables (biomass) were varied randomly within the range defined as the mean ± 95% confidence intervals of empirically derived values (Table 5.3).

Trace metal	Mean τ_M	SD	Min. τ_M	Max.τ_M	Median τ_M
Gadolinium	66	43	30	940	52
Zinc	32	21	16	150	27
Cadmium	29	32	11	225	21

5.4.4 Predicting the Effect of an Altered Microbial Food Web Structure on Trace Metal Residence Times

The effect of microzooplankton grazing on trace metal residence times and the chemical fractionation of trace metals in the aqueous phase was demonstrated by varying the proportions of plankton biomass. In these scenarios, the total biomass concentration was maintained at a constant level and the biomass of each plankton size classes was varied. Picoplankton were varied from 15 to 70% of the total plankton chl-*a* biomass; the total nanoplankton and microplankton biomass was adjusted appropriately, keeping the ratio of nanoplankton to microplankton biomass constant (Table 5.7). Plankton biomass state variables were varied according to the hypothesis that a shift in community structure due to reduced phosphorus loading will increase the dominance of picoplankton biomass while the ratio of microplankton to nanoplankton biomass remains constant. Changes in plankton size class composition are expected with a reduction in trophic status of surface waters (Stockner 1991). A reduction in Lake Erie phytoplankton biomass (nanoplankton and microplankton) in Lake Erie over the period from 1970 to 1987 (Makarewicz 1993) has been attributed to the ongoing reduction in total phosphorus loadings into the lake. Unfortunately, changes in Lake Erie picoplankton biomass during this time period are undocumented. However, the mean total chl-*a* measurements made during the 1994 and 1995 cruises (2.6 ± 1.5 $\mu g \cdot L^{-1}$; Table 5.3) are similar to the 2-4 $\mu g \cdot L^{-1}$ values reported for the pelagic central basin of Lake Erie during the summer months of 1979 (Charlton et al. 1993). Such a similarity suggests that total plankton biomass may

Table 5.7. Trace metal residence time of Gd, Cd and Zn in the surface water of Lake Erie under various regimes of plankton biomass composition. Total chlorophyll-*a* = 2.56 $\mu g \cdot L^{-1}$. The dynamic plankton model assumes steady-state plankton biomass. The average proportion of picoplankton in the Lake Erie surface water measured during summer months was 35%.

	Phytoplankton Biomass (μg chl-*a*$\cdot L^{-1}$)			Trace Metal Residence Time (days)		
Percent Picoplankton	**Micro**	**Nano**	**Pico**	**Gd**	**Zn**	**Cd**
15	0.71	1.47	0.38	45	22	21
35	0.54	1.13	0.90	47	24	19
50	0.42	0.86	1.28	49	25	18
70	0.25	0.52	1.79	53	27	16

have remained constant in pelagic waters during the recent decrease in phosphorus inputs into Lake Erie, and thus, a shift towards picoplankton productivity may have occurred, as hypothesized by Stockner (1991) and Weisse (1991).

Under the conditions of variable importance of picoplanktonic biomass, the dynamic model simulations predicted a slight increase in τ_M for Gd and Zn, whereas τ_{Cd} was inversely proportional to the percentage of picoplankton biomass (Table 5.7). This modeling result is attributed to the greater scavenging potential of the rapidly growing picoplankton for Cd versus Zn relative to the scavenging potentials for the other size fractions (Table 5.3).[1] The ecological significance of these results, in light of recent evidence supporting the notion of Zn limitation in Lake Erie plankton during thermal stratification (Nriagu et al. 1996) remains to be deciphered.

5.5 GENERAL DISCUSSION

The use of ecologically meaningful size fractions has enabled us to take into account the relative importance of trace metal scavenging by each particle size class in the microbial food web. Moreover, it has allowed us to establish causal links between the ecological fate of particles and the geochemical fates of their associated trace metals. Scavenging of trace metals by plankton can now be interpreted within the framework of plankton community dynamics.

[1] Since the model is based on microzooplankton regeneration of trace metal from picoplankton, the basic assumption of the model, i.e. that microzooplankton regenerate trace metals from picoplankton and affect the size distribution of trace metal in the aqueous phase, becomes increasingly relevant with an increase in the abundance of picopolankton. However, there is no reason to expect that trace metal regeneration from picoplankton by microzooplankton will be the same as that regenerated from nanoplankton and microplankton by mesozooplanktonic grazers. Thus, the present model has limited applicability since the relative importance of regeneration from other planktonic size fractions is not directly addressed in the model.

The activity of the microbial food web must be incorporated in any description of trace metal fates in the surface waters of Lake Erie during thermal stratification. The biota comprising this microbial community are a significant component of the suspended particulate matter, and this particle population undergoes continuous changes. Since the fate of trace metals is strongly linked to that of particulate matter, the activity of the microbial food web will have a significant influence on trace metal fates–through the scavenging of trace metal by newly produced biotic surfaces, transfer of this scavenged metal during the trophic transfer of biomass, and through remineralization processes such as trace metal regeneration by microzooplankton resulting from the ingestion and digestion of trace metals associated with prey items.

A Comparison of Trace Metal Residence Times Estimated by the Various Models – The level of agreement among the estimates of epilimnetic trace metal residence times in Lake Erie from the mass balance, static plankton, and dynamic plankton models is heartening (Table 5.8). Considering that the concentrations of Cd and Zn predicted by the dynamic model are derived from estimations based on the empirical relationships of trace metal partitioning within the microbial food web, the closeness of the real dissolved trace metal concentrations (Coale and Flegal 1989, Nriagu et al. 1996) to those predicted by the model gives credence to the ideas underlying the model. Hence, the dynamic model is considered to be a useful tool for predicting how changes to the structure of the microbial food web in pelagic Lake Erie might affect trace metal concentrations.

The trends in τ_M predicted by the dynamic plankton model (Cs >> Gd > Zn, Cd; Table 5.8) are not in full agreement with the predicted trends originating from the static plankton model (Cs >> Cd, Zn > Gd; Table 5.8). Only the static plankton model consistently predicted τ_M to be inversely proportional to the surface reactivity of the trace metal cations, as expected (*cf.* Whitfield and Turner 1987, Fisher 1986).

Concerning the more particle-reactive elements, the estimates for τ_{Gd} derived from the static plankton model (τ_{Gd} = 4-8 d) are much shorter than those obtained from the dynamic plankton model ($\tau_{Gd} = 66 \pm 43$ d), whereas the ranges in residence times for the two modeling approaches overlap for Cd and Zn (Table 5.8). Considering the inherently greater particle reactivity of Gd compared to the other (bivalent) cations, the predictionsof the dynamic model are suprising. The particle reactivity of Gd is expected to resemble that of Pb, on the basis of the respective stability constants for the first hydrolysis product ($\log_{10} K_{M\text{-}OH}$) of these elements: Gd = 6.3, Pb = 6.4 (Martell and Smith 1993). Hence, similar residence times for these two non-essential elements might be expected in systems that are controlled by

scavenging. Indeed, based on measured surface concentrations of dissolved Pb (Coale and Flegal 1989, Nriagu et al. 1996), and annual atmospheric Pb fluxes to Lake Erie reported

Table 5.8. A summary of trace metal residence times in Lake Erie surface water estimated from various steady-state models.

Model		Trace Metal Residence Time (days) Cs	Cd	Zn	Gd
I.	Mass balance				
	Data from:				
	Coale and Flegal 1989	---	230	94	---
	Nriagu et al. 1996	---	87	180	---
II.	Static plankton	510 - 760	13 - 41	10 - 28	4-8
III.	Dynamic plankton	514	29 ± 32	32 ± 21	66 ± 43

by Nriagu et al. (1996), τ_{Pb} is estimated to be 8-29 d. Based on the techniques used here to measure scavenging, and thus predict residence times using the dynamic plankton model, we are led to believe that strong competition for Gd between aqueous ligands and surface ligands exists in the surface water of Lake Erie during stratified conditions, and that complexation serves to keep this particle-reactive element in the aqueous phase. To assess the ability of the dynamic plankton model to predict residence times for Gd and other rare earth elements in the Great Lakes, further research will be required on the concentrations of these elements in the aqueous and particulate phases, and on their atmospheric and fluvial loading rates.

Limitations of the Plankton-Based Models – The trace metal residence times estimated by the plankton-based models presented here are season-specific. For example, one limitation to the use of VCFs in the static plankton model to predict τ_M is that VCF estimations are sensitive to changes in the concentration and speciation of dissolved metal. Possible errors in the use of VCFs derived from laboratory studies to estimate τ_M in the oceans may be less pronounced than in fresh waters, due to the relatively stable chemical conditions in most areas of the open ocean. However, in the surface waters of Lake Erie, where trace metals are expected to undergo dramatic changes in both total concentration and

speciation as thermal stratification is established in the spring, the use of laboratory-derived VCFs may not be as straightforward as in marine systems.

The method for estimating trace metal residence times in the dynamic plankton model highlights an important consideration: chemical analysis of "dissolved" trace metals in surface water will measure both colloidal and freely dissolved metals, yet these two forms of metal undoubtedly have different residence times in surface waters. The dynamic plankton model presented here considers $\tau_{Mp} << \tau_{M\text{-coll.}}$ (Equation 5.08). Some modeling efforts have accounted for the loss of colloidal metal. In their modeling of Th residence times in coastal marine environments, Baskaran et al. (1992) consider the majority of $^{234}Th^{4+}$ produced by the radioactive decay of ^{238}U, present in the form of a soluble uranyl carbonate complex, to be rapidly complexed by organic colloids. The fate of the colloids is considered to control the fate of the Th, and the authors invoke a "colloidal pumping" hypothesis wherein small colloids aggregate to form a larger colloid *cum* particle which eventually becomes sedimentable. This "pumping", or removal of metal from the surface water, is considered to be a physicochemical process. However, not all natural dissolved organic matter (NDOM) in surface waters has the same residence time. Considering that NDOM readily sorbs to biological surfaces (Campbell, Twiss and Wilkinson; unpublished data), and that the abundant cell surface of the picoplankton is rapidly incorporated into larger particles by microzooplankton grazing activity, it may well be that the microbial food web is not only a major contributor to NDOM, as mentioned by Baskaran et al. (1992), but also a major driving force in the conversion of dissolved colloidal metal into sedimentable matter. In the dynamic plankton model presented here, no attempt has been made to account for the loss of colloidal metal. Information regarding the distribution of trace metals between the labile and colloidal pools is very important since a significant fraction of regenerated Zn is excreted by microzooplankton as a complex >5 kD (presumably colloidal) and the precise fractionation of Gd in the aqueous phase of lake water is not certain. Further field investigations into the role of the microbial food web in determining the fate of colloidal trace metals in freshwater environments, as well as more information on seasonal changes in dissolved trace metal concentrations and trace metal speciation, are required.

Microbial Food Web Interactions with Trace Metals during the Onset of Stratification – Since the spring and summer are the periods of the greatest primary productivity, it follows that the majority of trace metal is likely removed from the water column during this period. The following scenario describes a hypothetical model of trace metal flux in pelagic Lake Erie during the establishment of the thermally stratified water column:

The loss of dissolved trace metal from the dissolved phase in the spring is probably due to sedimentary loss of diatoms following bloom conditions, as demonstrated in other lakes (Baliestrieri et al. 1992, Reynolds and Hamilton-Taylor 1992, Shafer and Armstrong 1991). However, since the spring bloom follows shortly after the onset of isothermal conditions, it is possible that relatively high concentrations of dissolved trace metals remain in the water column[2]. The subsequent onset of thermal stratification effectively isolates surface waters (Fig. 5.1) and scavenging of trace metals by rapid plankton growth in the warm epilimnetic waters continues to strip particle-reactive and bioactive trace metals from the dissolved phase. A shift towards increased productivity in the microbial food web would coincide with a period of intense scavenging, such as the scavenging witnessed during the months of July and early September (Chapter Four).

The results of the dynamic plankton model support the hypothesis of Zn limitation by plankton in the pelagic surface waters of Lake Erie during the summer. Nriagu et al. (1996) report a pronounced depletion of dissolved Zn in the epilimnion of Lake Erie during thermally stratified conditions and hypothesize that the loading rate of Zn into this lake is unable to supply biological demand for this micronutrient. Consistent with the hypothesis of Zn-limited plankton biomass in these waters at this time, the trophic transfer of Zn and Cd from radiolabeled picoplankton into nanoplankton and microplankton biomass was three-fold greater for Zn than Cd (Chapter Three). The brief residence time for Zn (τ_{Zn} 32 ± 21 d) predicted by the dynamic plankton model leads to low estimated concentrations of dissolved Zn prevailing in pelagic Lake Erie surface waters, as has been observed (Coale and Flegal 1989, Nriagu et al. 1996). In combination with increased complexation from dissolved ligands at the elevated prevailing pH of these waters (pH 8.1-8.9; Table 4.1), a very low concentration of bioavailable zinc would exist. These factors may combine to produce Zn-limited plankton biomass. It is intriguing to speculate that the balance between scavenging and regeneration by the microbial food web in the surface water of Lake Erie during thermally stratified conditions controls biological activity through the limitation of biologically essential trace elements.

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[2] Seasonal measurements of dissolved trace metal concentrations in surface waters are needed to confirm the assumption that relatively elevated concentrations of dissolved trace metals might persist after the onset of thermal stratification, such as expected if phytoplankton blooms produce abundant strong trace metal binding ligands. Activity of ^{137}Cs in material collected by sediment traps placed beneath the thermocline in Lake Michigan revealed that ^{137}Cs levels remained close to isothermal levels for several months after stratification was established (Robbins and Eadie 1991): a similar pattern might be expected for more particle-reactive elements, but the lag period would be expected to be much shorter.

CHAPTER SIX

CONCLUSION AND OTHER THOUGHTS

The logical basis for this research may be summarized as follows:

The microbial food web, characterized by the highly productive picoplankton and the intense grazing activity of microzooplankton, should play an important role in determining the fate of particle-reactive trace metals in surface waters. Rapid production of biomass surfaces leads to the efficient scavenging of trace metals, and the equally rapid consumption of these surfaces ultimately directs these trace metals towards larger sized particles. The loss of sedimentable particles is the main mechanism that controls the concentrations of trace metals in surface waters. However, due to digestive inefficiency, the intense grazing activity of the microzooplankton should also lead to appreciable regeneration of the trace metals previously scavenged by their prey. Non-reducible particle-reactive metals are likely to be *less* available for scavenging reactions following regeneration, in comparison with the form of these metals initially scavenged by the plankton. Thus, microzooplankton grazing is expected to increase the residence times of these elements in surface waters–in a manner that is currently unaccounted for by scavenging models based solely on the sorptive loss of metal to particle surfaces.

In this dissertation, I tested the hypothesis that microzooplankton can regenerate significant amounts of trace metal into the dissolved phase through the incomplete assimilation of trace metals from their prey during digestion. The hypothesis was tested using a combination of controlled laboratory studies, field studies, and mathematical models of empirical microbial food web interactions with trace metals.

The simplified laboratory model of the mixotrophic nanoflagellate *Ochromonas* grazing radiolabeled *Synechococcus* confirmed the hypothesis by demonstrating that a microzooplankter can indeed regenerate ^{137}Cs ^{109}Cd, ^{65}Zn and ^{153}Gd into the dissolved phase during the digestion of prey. Furthermore, the trace metal regeneration phenomenon was verified in natural waters sampled from the pelagic epilimnion of Lake Erie.

The hypothesis leads to the corollary that the grazing activity of microzooplankton will increase the residence time of those trace metals in surface waters that are scavenged by the prey of microzooplankton. The corollary was tested by incorporating the observed behaviours of both trace metals and the organisms within the microbial food web into a dynamic model. The model confirmed the corollary–microzooplankton grazing activity has a profound impact on trace metal residence times.

It is concluded that the geochemical fate of particle-reactive trace metals in the pelagic epilimnion of Lake Erie is closely linked to the activity of the planktonic community in this environment. This study is relevant to attempts to model trace metal fates in surface waters (*cf.* Santschi 1988). Although the ultimate fates of particle-reactive trace metals are controlled by scavenging and the sedimentary loss of particles, differences among metals in the regeneration, recycling, and trophic transfer within the microbial food web serve to partition these elements in a manner that is not entirely predictable from the metals' thermodynamically predicted surface reactivities. For example, under a scenario of increasing dominance in picoplankton biomass, the residence time of Cd is predicted to decrease, whereas the residence times of the other particle-reactive elements, Gd and Zn, increase (Chapter Five). Clearly, the trace metal regeneration hypothesis will serve to refine existing trace metal scavenging models.

The trace metal regeneration hypothesis is testable in many aquatic environments. Undoubtably it is present wherever the microbial food web exists, but it is likely to be most important where (and when) this trophic activity dominates, such as in mesotrophic and oligotrophic surface waters during summer months (Stockner 1991, Weisse 1991).

Hypotheses regarding the trace metal nutrition of plankton during thermal stratification can be addressed within the construct of the trace metal regeneration hypothesis. Observations consistent with Zn limitation of nanoplankton and microplankton (Chapter Three), and data gathered by Nriagu et al. (1996), suggest that trace metal limitation of plankton might occur in the pelagic epilimnion of Lake Erie during summer. Regeneration of limiting nutrients from prey will increase the chance for recycling of these nutrients by nutrient-limited biomass. Microzooplankton were inferred as the causative agents in regenerating iron from picocyanobacteria added to water sampled from a high nutrient, low chlorophyll (HNLC) region of the equatorial Pacific ocean (Hutchins et al. 1993) where Fe availability limits phytoplankton biomass. Increased Fe bioavailability following the consumption of recalcitrant Fe(III)-colloids by microzooplankton in the laboratory confirmed that regeneration could indeed play a significant role in alleviating Fe limitation in HNLC regions (Barbeau et al. 1996)[1]. Since the ecological fate of plankton and geochemical fate of trace metals are so closely linked, the seasonal

[1] Note the distinct difference in the effect that microzooplankton grazing has on increasing Fe availability for internalization by plankton (Barbeau et al. 1996) versus the reduced bioavailability of regenerated Zn measured over a 5 h period (Chapter Two). In the case of Fe, the reduction of the element that occurs during digestion serves to increase its availability for scavenging by internalization into cells versus oxidization of the element on the surface of cells or debris. Hence, through internalization into plankton biomass the residence time of Fe is increased. On the other hand, the complexation of Zn by organic ligands following excretion from microzooplankton increases the colloidal pool of this element. This will also increase its chance of recycling into plankton biomass since the residence time of colloids is much greater than that of particles and thus the complexed Zn will establish an equilibrium with more labile forms of Zn.

evolution of the plankton community and trace metal bioavailability are most likely just as closely related.

Regeneration of trace metals by microzooplankton is consistent with other well-established hypotheses, such as the regeneration of the macronutrients N and P by microzooplankton (Caron and Goldman 1990). In fact, the microbial loop hypothesis is grounded on the regeneration and recycling within the microbial food web of reduced carbon from autotrophic producers to heterotrophic bacteria by microzooplankton (Azam et al. 1983, Ducklow 1983). On the basis of the Redfield model of element stoichiometry in plankton biomass, the regeneration of trace metals from prey biomass by microzooplankton (Hutchins et al. 1993, Hutchins and Bruland 1994, Twiss and Campbell 1995, Twiss et al. 1996) and mesozooplankton (Hutchins and Bruland 1994, Wang et al. 1996) is expected.

The phenomenon of trace regeneration by microzooplankton is reasoned to apply equally to the regeneration of other environmentally significant trace solutes from plankton such as vitamins, plant hormones (e.g. cyclic adenosine monophosphate; Francko 1989), dimethylsulfide (Sunda 1995), and hydrophobic organic contaminants (Broman et al. 1996). In addition, the production of recalcitrant organic matter arising from microzooplankton grazing activity (Tranvik 1994) is likely an important source of trace metal complexing ligands (*cf.* Section 3.4.4) that may be present at sufficient concentrations to influence the speciation and thus, bioavailability of some trace metals in surface waters (Sunda 1995). Extensive reworking of organic carbon in surface waters, evident from enhanced $\delta^{13}C$ levels in sediments dating from the Proterozoic period (2,500-540 million years ago) is attributed to the absence of organisms that produced rapidly settleable feces (Logan et al. 1995). Given the evidence presented here for the degree of trace metal regeneration within the microbial food web, trace metal cycling in oceanic surface waters during this pre-feces era may have influenced the evolution of the physiological requirements and responses of plankton to trace metals.

APPENDIX A

LOSS OF ^{153}GD AND ^{65}ZN FROM PHYTOPLANKTON BIOMASS IN THE PRESENCE OF EDTA

A.1 PURPOSE

Loss of ^{153}Gd from the picoplankton (0.2-3 μm) size fraction in control cultures was observed in all three replicates of the grazing experiments described in Chapter Two and during a preliminary experiment, despite a preliminary cell wash using 10^{-4} EDTA for 15 minutes. The purpose of this assay was to closely observe the loss of this element in an attempt to discover a reason for the retarded removal of ^{153}Gd from *Synechococcus* biomass.

A.2 METHODS

Synechococcus was exposed to ^{153}Gd and ^{65}Zn for 24 h, as described in Section 2.3.1. Twenty mL of radioactive culture was removed and filtered onto a superimposed 0.2-μm polycarbonate membrane filters (Nuclepore) as described in Section 2.3.2, then rinsed with 5 mL of non-radioactive culture medium. An aqueous solution of Na_2EDTA was then added directly into the radioactive culture medium to achieve 10^{-4} M EDTA. At timed intervals (7 to 106 min), 20 mL of radioactive culture was filtered onto superimposed filters, and rinsed (*as described above*).

A.3 OBSERVATIONS AND COMMENTS

A slight loss of ^{65}Zn occurred within the first time interval (7 min; Fig. A) and little further loss of Zn occurred; this is consistent with the low fraction of ^{65}Zn sorbed to the cell surface (<5%) after a 24 h exposure (Table 1.2). In contrast to ^{65}Zn, a significant fraction (≈50%) of the total cellular ^{153}Gd was lost during the initial 7 min exposure to EDTA. After 106 min, only ≈25% of the original total ^{153}Gd was retained by the *Synechococcus* biomass. Rapid removal within 7 min may correspond to an easily exchangeable surface-bound fraction of ^{153}Gd, whereas the slower loss over the following 99 min corresponds to the extraction of ^{153}Gd from intercellular/intramembaneous pools.

The data suggest that Gd is tightly-bound by *Synechococcus*. Several hypotheses can explain the slow removal rate of ^{153}Gd from the cell, relative to ^{65}Zn. Since the Gd^{3+} cation is a moderate substitute for native Ca^{2+} and Fe^{3+} in metalloproteins (Hughes 1985, p. 68) it follows that ^{153}Gd may be

involved with Ca- and Fe-specific ligands present in *Synechococcus* biomass. It is possible that ^{153}Gd may be bound by a surface ligand with a higher affinity for Gd than EDTA (K_{GdEDTA} = 17.32, μ = 0.1; Martell and Smith 1993), such as a siderophore-type ligand. Alternatively, if the kinetics of the reaction, $\equiv L\text{-}Gd \rightarrow \equiv L^{3-} + Gd^{3+}$, are slow then the surface ligand ($\equiv L$) could possess a lower affinity for Gd^{3+} than EDTA.

The pattern of ^{153}Gd loss from *Synechococcus* is similar to the loss of the actinide ^{241}Am from the marine diatom *Thalassiosira pseudonanna* and the marine chlorophyte *Dunaliella tertiolecta* (Fisher et al. 1983): two cellular pools of ^{241}Am were observed–an easily exchangeable pool and tightly-bound one, the percentage of the latter increasing with exposure time (as observed for ^{153}Gd accumulation by *Synechococcus*; Fig. 2.1)–and the majority of ^{241}Am was associated with cell walls and membranes. Although treatment with the sulfhydryl-complexing reagent ß-mercapto-ethanol revealed that ^{241}Am was not binding to -SH groups in proteins (Fisher et al. 1983), this does not exclude the possibility of metal-substitution in metalloproteins by ^{153}Gd, as hypothesized here, despite the fact that these two trivalent elements appear to have similar patterns of accumulation.

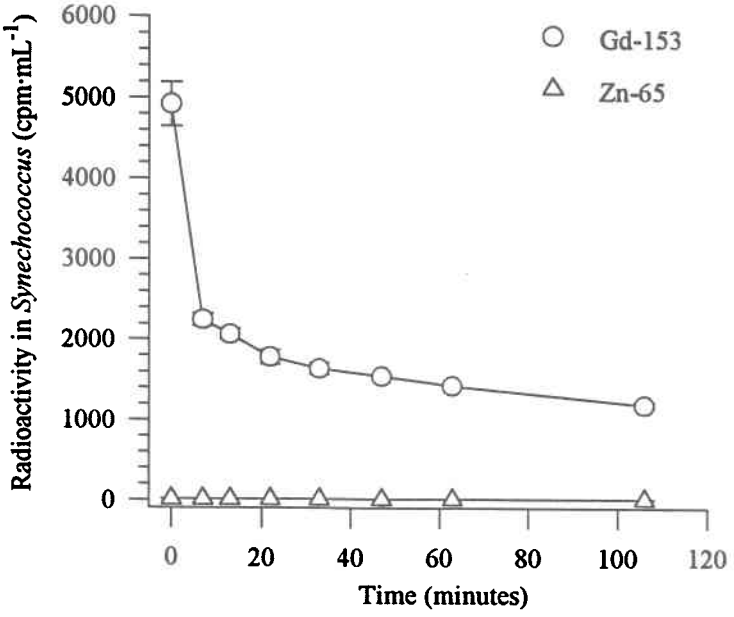


Figure A. Loss of ^{153}Gd and ^{65}Zn from biomass of *Synechococcus leopoliensis* over time in the presence of 10^{-4} EDTA. Values are mean ± SD, n = 3.

APPENDIX B

VERIFICATION OF THE SERIAL GRAVITY FILTRATION TECHNIQUE USED FOR SIZE-SELECTIVE FRACTIONATION

B.1 PURPOSE

The following tests were carried out to determine if the filtration technique used for size-selective fractionation of particles and the isolation of the dissolved phase (<0.2 m) produced artifacts such as: the enhancement of trace metals in the dissolved phase due to cell lysis during filtration, or breakage of fragile cells during the rinsing step. In addition, the precision of a serial filtration technique was compared to that of a parallel filtration technique.

B.2 METHODS

A grazing experiment was conducted, as described in Sections 2.3.1 and 2.3.3, with the exception that only ^{109}Cd and ^{65}Zn were used for radiolabeling *Synechococcus*. After 46 h, the size fractionation of radionuclides was determined in the grazing and control treatments. Size fractionation was conducted by reverse and gravity filtration (Fig. B). In addition, the effect of rinsing on the retention of radionuclides in the >3 µm-size fraction was determined. All filtration protocols treatments were conducted in triplicate. Samples for total aqueous radioactivity and filtrates <0.2 µm were collected in duplicate per filtration treatment replicate. All gravity filtrations were conducted with <13 kPa of vacuum pressure.

B.2.1 Reverse Filtration

Twenty mL of culture (***c***) was gravity-filtered onto a 0.2-µm filter (***A***). Filtrate (<3 µm) was collected by immersing a pre-dampened 3-µm filter device (***b***) into the culture medium (***c***); filtration proceeded by hydrostatic pressure (<1 cm head). Filtrate (<3 µm) was then gravity-filtered onto a 0.2-µm filter (***B***) and the filtrate <0.2 µm (***C***) was collected. Further fractionation of the <0.2 µm-filtrate was conducted using an ultrafiltration membrane filter to produce filtrate <5 kD (***D***).

After the filtrates were removed, filters ***A*** and ***B*** were rinsed with 5 mL of FRAt prior. The radioactive content of the fraction >3 µm was determined using reverse filtration by repeating the filtration protocol but by omitting the rinsing step for filters ***A*** and ***B***. Background 0.2-µm filter blanks

for non-rinsed filters were established by re-filtering 0.2-µm filtered radioactive culture through 0.2-µm filters, and removing the filters from the filter holder without a rinse step.

Radioactivity in each size fraction (Fig. B.1; cpm·mL^{-1}) was determined as follows:

Fraction	Grazing Treatment
>3 µm	= ***A*** - ***B***
0.2-3 µm	= ***B***
<0.2 µm	= ***C***
<5 kD	= ***D***

B.2.2 Serial Gravity Filtration

Filtration was conducted as described in Section 2.3.3.

Radioactivity in each size fraction (Fig. B.1, B.2; cpm·mL^{-1}) was determined as follows:

Fraction	Grazing Treatment	Prey Control Treatment
>3 µm	= ***E***	---
0.2-3 µm	= ***F***	= ***I***
<0.2 µm	= ***G***	= ***J***
<5 kD	= ***H***	= ***K***

B.2.3 Parallel Gravity Filtration

Filtration steps were conducted as described in Section 2.3.3. with the exception that filtrate <3 µm was not filtered onto a 0.2-µm filter. Instead, the radioactivity (Fig. B.1, cpm·mL^{-1}) in the size fraction 0.2-3 µm was determined by filtering whole culture directly onto a 0.2-µm filter; radioactivity in the fraction 0.2-3 µm was determined by the method of difference as follows:

Fraction	Grazing Treatment
>3 µm	= ***E***
0.2-3 µm	= ***A*** - ***E***

B.3 RESULTS

B.3.1 The filtration efficiency of reverse versus gravity filtration

In a preliminary test of the reverse filtration device (***b***, Fig. B.1), the retention of *Ochromonas* by gravity and reverse filtration through a 3-µm filter was compared. Cell density of the intact cells of *Ochromonas* and vesicles (chrysolaminarin vesicles and large pieces of similar sized debris (≈2 µm

dia.) was determined by optical microscopy. Similar cell and vesicle densities were determined in filtrate produced by the two methods.

The filtration efficiency of the whole culture by gravity filtration was 96.8% and 99.7% by reverse filtration. Reverse filtration was an order of magnitude more efficient in retaining intact cells, and 3-4 times more efficient at preventing cell lysis and/or retaining vesicles.

Culture Fraction	*Ochromonas* (cells·mL^{-1})	Vesicles (·mL^{-1})
Whole medium	$1.16 \times 10^5 \pm 6.90 \times 10^3$	$3.30 \times 10^3 \pm 1.50 \times 10^3$
Filtrate <3 μm, gravity filtration	$3.67 \times 10^3 \pm 3.77 \times 10^3$	$4.17 \times 10^3 \pm 3.54 \times 10^3$
Filtrate <3 μm, reverse filtration	$0.33 \times 10^3 \pm 0$	$1.34 \times 10^3 \pm 0.47 \times 10^3$

(Cell counts of whole culture and filtrates were conducted in triplicate; values for filtrate are the mean of duplicate filtrations.)

B.3.2 The effect of gravity versus reverse filtration on radionuclide content in the dissolved phase

If lysis of *Ochromonas* was occurring on the 3-μm filter, then trace element regeneration by grazing might be over-estimated due to filtration-induced lysis of the grazer; the results of the comparison of radionuclide content in the filtrate <0.2 μm from the grazing treatment produced using reverse and gravity filtrations confirm that this was not occurring. There was no significant difference (P <0.05) in the amount of radionuclides present in the filtrate <0.2 μm obtained using gravity or reverse filtration (*see data below*). Although the reverse filtration produce less ^{65}Zn in the fraction <5 kD (as expected if less cell lyses was occurring), the difference was not significant. This supports the claim that the gravity filtration technique used routinely in the laboratory study did not overtly enhance cell lysis of *Ochromonas* and positively bias the measurement of trace metal regeneration.

		Radioactivity <0.2 μm	
Treatment	Filtration	^{109}Cd (cpm·mL^{-1})	^{65}Zn (cpm·mL^{-1})
Prey Control	Gravity	131 ± 2	62 ± 3
Grazing Treatment	Gravity	173 ± 5	45 ± 6
Grazing Treatment	Reverse	168 ± 3	45 ± 2

		Radioactivity <5 kD (%<0.2μm)	
Treatment	Filtration	^{109}Cd (cpm·mL^{-1})	^{65}Zn (cpm·mL^{-1})
Prey Control	Gravity	99 ± 11 (76 ± 9%)	14 ± 10 (23 ± 16%)
Grazing Treatment	Gravity	142 ± 15 (82 ± 9%)	22 ± 21 (47 ± 46%)
Grazing Treatment	Reverse	130 ± 13 (77 ± 8%)	10 ± 8 (21 ± 18%)

(Values are mean ± SD, n = 3.)

The amount of ^{109}Cd (<0.2 μm) in the grazing treatment was greater than in the prey control treatment, consistent with the hypothesis of regeneration from the particulate phase due to grazing activity. However, the amount of dissolved ^{65}Zn in the prey control treatment was greater than that observed in the grazing treatment which is inconsistent with the results of all the previous 3 replicates of this experiment (*see* Chapter Two). Nevertheless, the comparison of gravity versus reverse filtration on the amount of dissolved radionuclides within the grazing treatment remains valid.

B.3.3 The effect of filter rinsing on the accumulation of radionuclides in the fraction >3 μm

Rinsing filters with 5 mL of non-radioactive culture medium reduced the radionuclides retained by the filters, regardless of the filtration technique used. Losses due to rinsing were identical between filtration schemes: 30% loss of ^{109}Cd, 26-24% loss of ^{65}Zn. In the case of ^{109}Cd, there was no significant difference between gravity and reverse filtration within rinse treatments. However, gravity filtration produced a greater apparent loss of ^{65}Zn relative to reverse filtration for both rinsing and non-rinsing protocols. This is not attributed to an enhanced loss of ^{65}Zn from cells lysing due to gravity filtration–if this were the case then one would expect a concomitant loss of ^{109}Cd. Instead, it is likely that the capture of *Ochromonas* on the 0.2-μm filter (***A***) also retained a significant amount of 0.2 μm-filterable ^{65}Zn, probably present as waste ejested from the cell yet remaining adhered to the cell surface (as seen while observing exocytosis from *Ochromonas* using optical microscopy).[1] It is concluded that this 0.2 μm-filterable material is lost during filter rinses, and during gravity filtration onto 3-μm filters.

[1] Some ^{109}Cd may also be associated with this 0.2 μm-filterable material however, the <5 kD size fractionation of ^{109}Cd indicates that most regenerated ^{109}Cd is consistently in a smaller size fraction that ^{65}Zn, an hence, less likely to aggregate on particle surfaces.

		Radionuclide content (cpm·mL^{-1}) in the >3 µm-fraction	
Filtration technique		^{109}Cd	^{65}Zn
Gravity	No rinse	63 ± 3	31 ± 1
	With rinse	44 ± 6	23 ± 4
Reverse	No rinse	70 ± 9	42 ± 4
	With rinse	49 ± 14	32 ± 6

(Values are mean ± SD, n = 3, except the error of the fraction >3 µm estimated by the reverse filtration technique was propagated from the SD of mean of ***A*** and ***B*** [Fig. B.1].)

B.3.4 The precision of serial versus parallel filtration

Sequential filtration was chosen to reduce error in measuring radionuclides in the 0.2-3 µm fraction. For example, in the sequential filtration protocol the amount of metal on the 0.2-µm filter was measured directly, i.e. the radionuclide retained on the filter. In a parallel filtration scheme the amount of metal in the fraction <3 µm but >0.2 µm must be determined by difference, i.e. by comparing metal retained directly by the 3-µm and the 0.2-µm filters. Hence, an additional error is incorporated into this estimation which must be propagated, i.e.

$$\text{Mean}_{0.2\text{-}3\,\mu\text{m}} \pm \text{SD} = \text{Mean}_{>0.2\,\mu\text{m}} - \text{Mean}_{>3\,\mu\text{m}} \pm \sqrt{(\text{SD}_{>0.2\,\mu\text{m}}{}^2 + \text{SD}_{>3\,\mu\text{m}}{}^2)}$$

where SD is the standard deviation of three replicate filtrations. In contrast, the sequential filtration approach measures the amount of metal directly:

$$\text{Mean}_{0.2\text{-}3\,\mu\text{m}} = \text{Mean}_{>0.2<3\,\mu\text{m}} \pm \text{SD}_{>0.2<3\,\mu\text{m}}$$

where the mean ± SD is derived from 3 replicate filtrations. The parallel filtration scheme provides the same results as those obtained from the sequential filtration approach. However, there is a greater degree of error in the estimate derived from parallel filtrations.

	Radionuclide content (cpm·mL^{-1}) in the 0.2-3 µm fraction	
Gravity Filtration Scheme	^{109}Cd	^{65}Zn
Sequential	104 ± 3	25 ± 1
Parallel	107 ± 15	30 ± 6

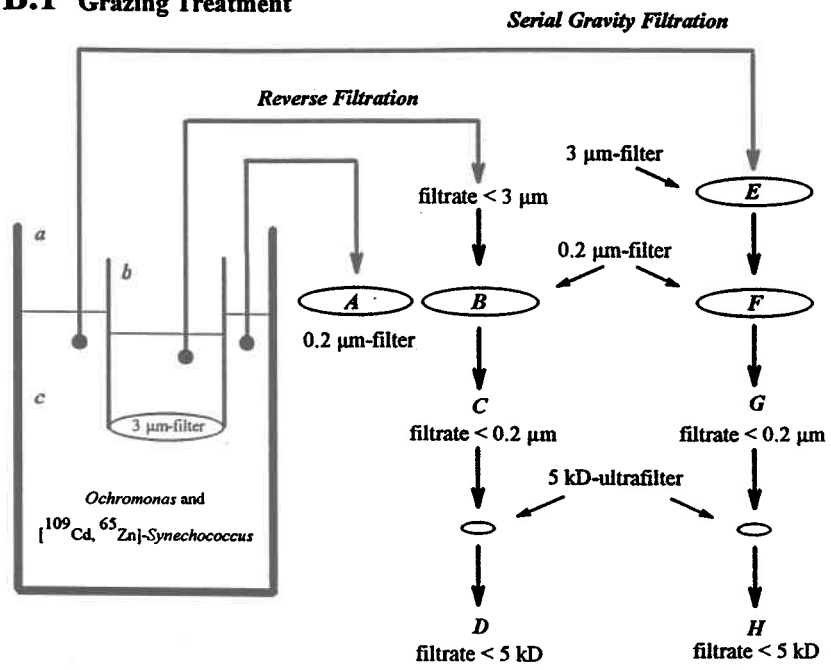
B.4 CONCLUSION

Reverse filtration was more efficient (99.7%) than gravity filtration (98.6%) for retaining *Ochromonas*, and reverse filtration provoked less apparent cell lysis. However, there was no significant difference in the level of radionuclides present in the dissolved phase isolated using either method.

Rinsing removed appreciable amounts of radionuclides from the cells of *Ochromonas*. The data suggest that a positive bias for the estimation of radionuclide retention by *Ochromonas* using reverse filtration may arise from the retention of 0.2 μm-filterable material, such as observed for ^{65}Zn but not for ^{109}Cd.

Parallel and serial gravity filtration gave identical results for the measurement of radionuclides present in the 0.2-3 μm size fraction, although the estimation based on the parallel filtration scheme had a much greater degree of error.

B.1 Grazing Treatment



B.2 Prey Control Treatment

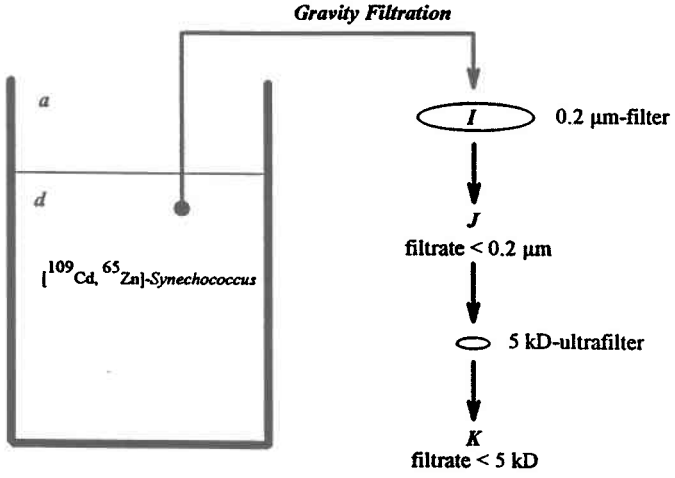


Figure B. Schematic diagram of the sampling and filtration protocols use to test for filtration artifacts during the size selective filtration of *Ochromonas* and *Synechococcus*. **1.** Grazing treatment, containing *Ochromonas* and *Synechococcus*. **2.** Prey control treatment, containing *Synechococcus* only. ***a***, silanized 300-mL borosilicate beaker; ***b***, 125-mL low density polyethylene cylinder with an attached 3-µm filter; ***c***, FRAt medium containing *Ochromonas* and radiolabeled *Synechococcus*; ***d***, FRAt medium containing radiolabeled *Synechococcus*. In order to avoid the concentration of particles >3 µm in the culture medium due to the reverse filtration scheme, culture was removed for the serial gravity filtration steps prior to immersing the reverse filtration device into the culture medium. With the exception of the ultrafilters, all filters were polycarbonate membrane filters (47 mm diameter; Nuclepore).

APPENDIX C

THE USE OF METABOLIC INHIBITORS TO ASSESS THE ABIOTIC SORPTION OF TRACE METALS BY LAKE PLANKTON

C.1 PURPOSE

Since trace metal scavenging by plankton is the net result of various abiotic (e.g. surface complexation) and biotic (e.g. active uptake) processes, an assessment of trophic transfer of trace metals from prey to predators in the plankton size class requires a measurement of the amount of metal that is scavenged by abiotic means alone. Ideally, the addition of metabolic inhibitors added to lake water at effective concentrations renders plankton equivalent to abiotic organic surfaces. Thus, the difference in scavenging in the presence and absence of metabolic inhibitors may then be attributed to biotic processes, which includes the consumption of metal laden prey during trophic transfers in the microbial food web.

Metabolic inhibitors were used to control for the relative contribution of surface sorption of ^{137}Cs, ^{109}Cd, ^{65}Zn and ^{153}Gd by seston from Lac Bedard, a small circumneutral lake, located in the Laurentian Mountains north of Quebec City.

C.2 METHODS

Water from the epilimnion of Lac Bedard was sampled from a depth of 1 m at 1100h on 26-09-93. Water was immediately filtered through a 210-µm polypropylene screen (Spectrum) and collected in acid-washed 4 L polypropylene jugs. Jugs were placed in a cooler and transported to the laboratory (1.5 h transit). In the laboratory, 950 mL of water from depth was dispensed into 4-1 L acid-washed Teflon bottles. Control treatments received one of two metabolic inhibitors or a ≈0°C treatment (*see below*). All treatments (except 0° C inhibited treatments) were placed in an environmental chamber (10.6 °C, 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a photoperiod of 12:12 set to match the date and latitude); treatments were allowed to equilibrate in the bottles for ≈1.5 h prior to adding the radioactive cocktail. The 0°C treatment bottle was chilled for 1.5 h in the dark before the addition of the radionuclides; this treatment remained under this condition for the duration of the experiment. Sodium azide (154 mM), and formaldehyde (170 mM) were added to separate bottles 45 min before the addition of the radioactive

cocktail; the pH of the stock solutions of these inhibitors were adjusted so as not to alter the ambient pH of the sampled lake water (pH 6.4).

Trace metal were added in a 0.5 M HCl matrix to give a nominal final concentration of 0.57 nM Cs, 12.1 nM Cd, 17.2 nM Zn, and 1.28 nM Gd. Immediately prior to adding the radionuclide spike to each bottle, an equivalent volume of 0.5 M NaOH was added (the radioactive spike followed within 2 sec of the addition of base). Bottles were mixed thoroughly by gentle inversions and sampled at 2, 5, 12, 25 h according to the protocol described in Chapter Three (Section 3.3.3) with the following changes: the microplankton was collected onto 12-μm filters instead of 20-μm filters, and superimposed filters were used for all filtrations (the bottom filter was used as a control to account for sorption of radionuclides by the filter). The control treatments (azide, formaldehyde, °C) were sampled only after 25 h.

C.3 RESULTS AND CONCLUSION

The phytoplankton biomass (μg chl-*a*·L^{-1}) in the lake water was: microzooplankton (12-210 μm), 3.92 ± 0.24; nanoplankton (2-12 μm), 1.24 ± 0.10; picoplankton (0.2-2 μm), 0.81 ± 0.15.

Accumulation of all radionuclides in the nanoplankton and picoplankton of living treatment reached an apparent plateau within 25 h (Figure C). The microplankton did not appear to have reached any saturation. Total radionuclide accumulation by the combined plankton size fractions (percentage of radionuclides added) in the living samples were ^{153}Gd (9.3%) >> ^{109}Cd (1.9%) > ^{65}Zn (1.1%) > ^{137}Cs (0.3%) (Table C).

The mean of the three inhibitors used generally had a lower amount of ^{109}Cd, ^{65}Zn and ^{153}Gd accumulation in a respective size fraction in comparison with the living treatment at depth (Table C). If the values for ^{137}Cs accumulation in the azide treatment are excluded then the same trend was visible for ^{137}Cs partitioning. The results for cesium accumulation suggest that Na from the sodium azide effectively out-competed ^{137}Cs ([^{137}Cs] = 0.6 nM) for uptake onto particles and hence, the azide treatment is not a valid control for measuring the accumulation of trace amounts of Cs by particles. The 0°C treatment would be considered to be the best inhibitor to use, with respect to the fact that it has creates no harsh chemical treatment of cellular material. Generally, in the presence of inhibitors, less ^{137}Cs, ^{109}Cd, ^{65}Zn and ^{153}Gd accumulated into the plankton in comparison with the living treatment. However, when metabolic inhibitors are used, interpretation of the observed scavenging behaviour is difficult since the value for partitioning in the presence of inhibitors refers to trace metal adsorbed to

particles which existed when the inhibitor was added–particle dynamics in a living sample are such that the relative importance of different particle populations may well vary over time, generating subtle differences between living and inhibited treatments.

Table C. Accumulation of radioisotopes in various plankton size fractions from surface waters of Lac Bedard, Quebec, after 25 h in the presence and absence of a metabolic inhibitor. Values are percentage of total radioactivity. Means (± SD) are calculated for all three inhibitors except in the case for ^{137}Cs, accumulation in the presence of NaN_3 is excluded. %CV = percent coefficient of variation.

Plankton	Treatment	^{109}Cd	^{153}Gd	^{137}Cs	^{65}Zn
Microplankton (12-210 µm)	Living	0.98	2.80	0.19	0.53
	NaN_3	0.22	2.33	0.001	0.44
	Formalin	0.79	2.95	0.04	0.43
	0°C	0.51	1.69	0.05	0.20
Nanoplankton (2-12 µm)	Living	0.42	2.62	0.04	0.26
	NaN_3	0.17	3.57	0.006	0.42
	Formalin	0.35	2.94	0.04	0.25
	0°C	0.29	1.92	0.04	0.11
Picoplankton (0.2-2 µm)	Living	0.48	3.92	0.03	0.36
	NaN_3	0.27	6.50	0.01	0.42
	Formalin	0.52	5.29	0.02	0.44
	0°C	0.47	4.71	0.03	0.29

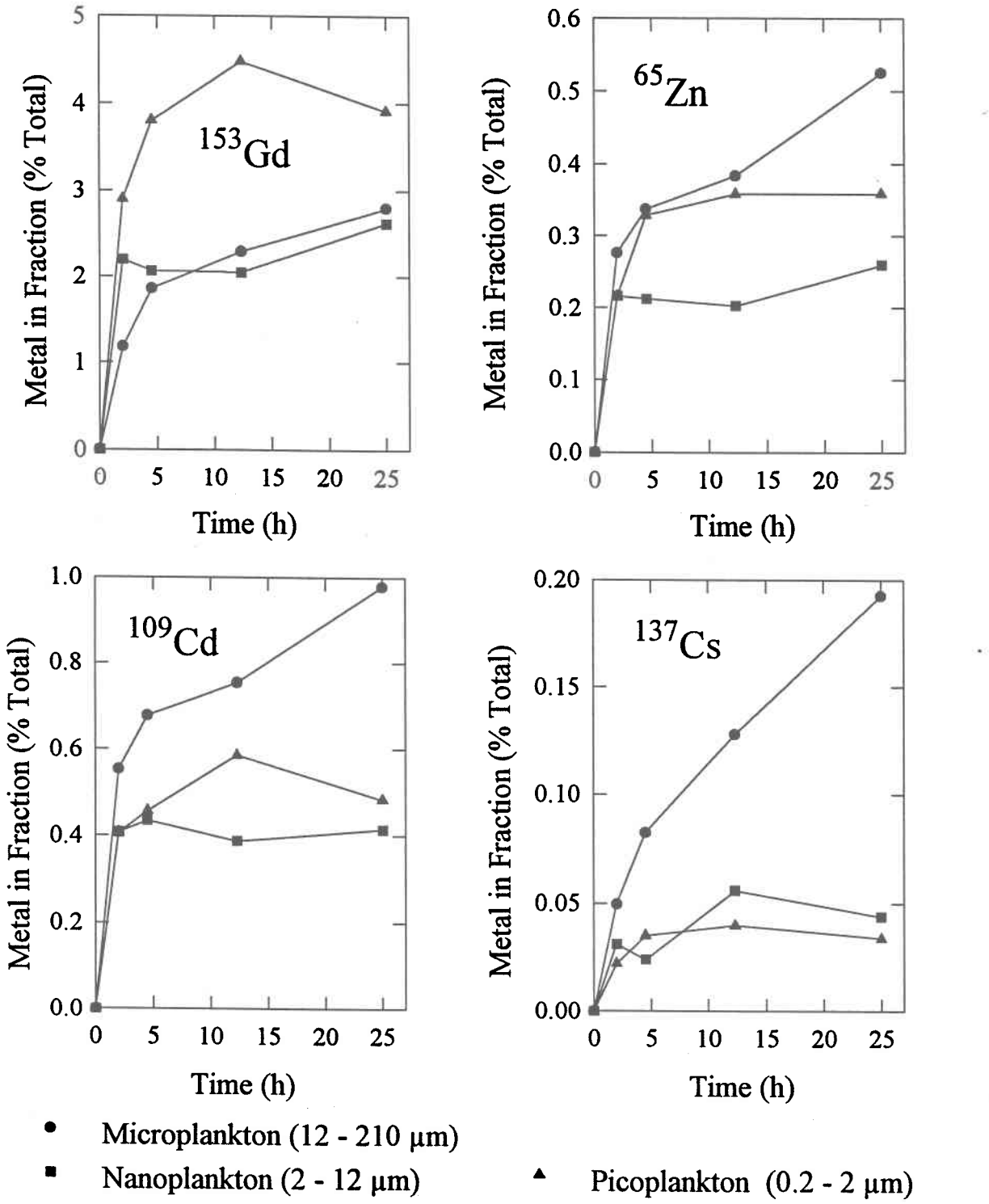


Figure C. Partitioning of ^{137}Cs, ^{153}Gd, ^{109}Cd and ^{65}Zn in plankton (<210 µm) from the epilimnion of Lac Bedard, Quebec. Nominal total metal concentrations were 0.57 nM Cs, 1.28 nM Gd, 12.1 nM Cd and 17.2 nM Zn.

APPENDIX D

RELATIONSHIPS BETWEEN THE ACCUMULATION OF TRACE METALS AND THE SURFACE AREA TO VOLUME RATIO OF FRESHWATER PHYTOPLANKTON: LITERATURE SOURCES

D.1 PURPOSE

The accumulation of particle-reactive trace metals from solution by phytoplankton is proportional to the surface area (A) of the organism exposed to solution. The concentration of the trace metal by the biovolume (V) of the organism is thus related to the ratio of biological surface area to volume (A:V). Studies that have examined the volume concentration factor (VCF) of numerous trace metals as a function of phytoplankton A:V demonstrate that the VCF increases with A:V in marine (Fisher and Reinfelder 1995) and freshwater (Stary et al. 1983b) phytoplankton. Moreover, these studies conclude that the VCF of trace metals is generally the same regardless of the phytoplankton species, i.e. A:V is more important than the different surface properties exhibited by various phytoplankton.

Since A:V is an important factor in determining the VCF of a given trace metal, the static phytoplankton model described in Section 5.3.2 must incorporate the different scavenging potentials for each plankton size classes, based on the relationship of VCF:(A:V). In contrast to marine studies (*cf.* Fisher and Reinfelder 1995), there is a paucity of studies that have systematically examined the relationship of VCF and A:V for freshwater phytoplankton. Nevertheless, several studies are available which are suitable for establishing a tentative relationship between the VCF and A:V for some of the trace metals examined in the present study.

D.2 METHODS

The criteria for choosing the studies listed below were as follows: exposure levels of trace metals were low (micromolar range, or less); no use of high affinity trace metal ligands (e.g. EDTA) in the exposure media; media pH 7-9; the use of living biomass; and availability of sufficient information to calculate a VCF. In cases where metal accumulation is reported as mass per unit dry weight, the dry weight to living plankton volume was estimated using the relationships reported by Nalewajko (1966) for chlorophytes, diatoms, and cyanobacteria. Cell volumes were estimated by reference to geometric descriptions of the species (Tiffany and Britton 1952).

D.2 RESULTS

The data gathered from published reports are listed in Table D. Not all of the data were chosen for analysis by simple linear regression (Fig. D). The VCFs calculated from the data of Hassett et al. (1981) are considered to be anomalously high for some species; although some of these values are close to the anticipated VCF values, the presence of some extremely elevated VCF values for Cd (e.g. $>10^6$) was the basis for rejecting the entire set. The technique employed by Hassett et al. (1981) may have induced the precipitation of Cd (VCFs were calculated from the loss of ^{109}Cd from the filtrate). The use of high phosphate in the exposure medium may have attributed to the very low VCF (VCF_{Cd} = 299, measured by accumulation in biomass) determined from the study of Gipps and Coller (1980).

The regressions used for estimating the VCFs of the plankton size classes in the static model are as follows: Cs: VCF = 80.2 (A:V) + 271; r^2 = 0.13, 12 d.f. (P >0.1)

Cd: VCF = 7,200 (A:V) + 14,605; r^2 = 0.13, 5 d.f. (P >0.1).

The VCF value for Zn was estimated from the correlation established from the data of Fisher (1985) for marine phytoplankton: VCF_{Zn} = 8,442 (A:V) - 5,705, r^2= 0.98, 3 d.f. (P <0.01). This relationship was observed in sea water where the concentration factor of Zn by phytoplankton biomass is expected to be less than in fresh water due to different chemical speciation. To correct for the different levels of accumulation, and assuming the slope of A:V versus VCF is constant regardless of the aqueous medium, the results of several determinations of VCF_{Zn} for *Synechococcus* ($VCF_{Zn} = 10^{4.84}$; Table D) were used to correct estimate the relationship for VCF_{Zn} in fresh water. The relationship becomes: VCF_{Zn} = 8,442 (A:V) + 32,100.

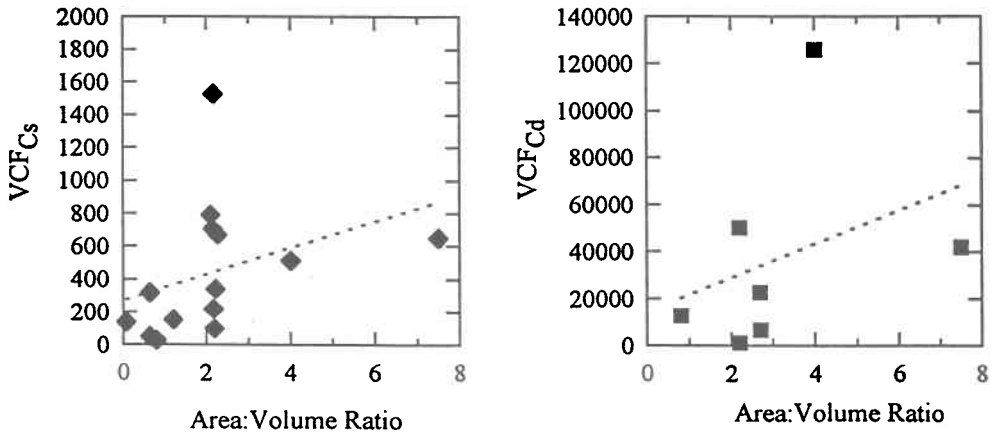


Figure D. The relationship between the concentration of cesium and cadmium from solution by phytoplankton and the surface area to volume ratio of the phytoplankton.

Table D. Reported volume concentration factors of Cs, Cd and Zn for freshwater phytoplankton.

Reference	Species	Area (A), μm^2	Volume (V), μm^3	A:V	log_{10} VCF: Cs	Cd	Zn
[1] King 1964	*Chlamydomonas* sp.	278	435	0.64	2.50	---	---
[2] Williams and Swanson 1958							
	Rhizoclonium hieroglyphicum	4,815	2,209	2.18	3.18	---	---
	Oedogonium vulgare	676	322	2.10	2.90	---	---
	Spirogyra ellipsospora	149,839	67,495	2.22	2.53	---	---
	Spirogyra communis	4,700	2,160	2.18	2.34	---	---
	Gonium pectorale	22,698	321,555	0.07	2.14	---	---
	Oocystis elliptica	961	424	2.27	2.83	---	---
	Chlamydomonas sp.	278	435	0.64	1.72	---	---
	Euglena intermedia	10,132	4,712	2.15	2.85	---	---
	Chlorella pyrenoidosa	79	65	1.20	2.19	---	---
[3] Conway and Williams 1979							
	Asterionella formosa	575	214	2.69	---	4.35	---
	Fragellaria crotonensis	515	191	2.69	---	3.82	---
[4] Cain et al. 1980	*Scenedesmus obliquus*	320	146	2.19	---	3.08	---
[5] Gipps and Coller 1980	*Chlorella pyrenoidosa*	79	65	1.20	---	2.48	---
[6] Hassett et al. 1981	*Navicula pelliculosa* (pH 7)	960	1,664	0.58	---	5.20	---
	" " (pH 8)					5.25	
	Chlorella pyrenoidosa (pH 7)	79	65	1.20	---	6.99	---
	" " (pH 8)					6.78	
	Scenedesmus obliquus (pH 7)	320	146	2.19	---	6.04	---
	" " (pH 8)					5.98	
	Nostoc sp.	87	38	2.25	---	5.65	---
	Oscillatoria sp.	145	63	2.31	---	5.60	---

Table D. (continued).

Reference	Species	Area (A), μm^2	Volume (V), μm^3	A:V	log_{10} VCF: Cs	Cd	Zn
[7] Stary and Kratzer 1982	*Chlorella kessleri*	177	221	0.80	---	4.10	4.50
[8] Stary et al. 1983a	*Chlorella kessleri*	177	221	0.80	1.45	---	---
	Scenedesmus obliquus	320	146	2.19	2	4.70	5.00
[9] this study[a]	*Synechococcus leopoliensis*	7.07	1.77	3.94	2.71	5.10	4.84
	Synechococcus sp.[b]	2.01	0.27	7.45	2.81	3.82	3.76

Note: The VCFs reported for *Synechococcus leopoliensis* are the mean of 4 measurements after various exposure durations (24-72h). [b] Lake Huron isolate LH #2 (gift from G. L. Fahnenstiel).

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