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Trophic transfer of Fe, Zn and Am from marine bacteria to a planktonic ciliate

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ABSTRACT: Marine bacteria may serve as an enriched source of essential and non-essential metals for animals that eat them. We evaluated this trophic transfer by measuring the accumulation of Fe, Zn and Am by planktonic ciliates (*Uronema* sp.) following the ingestion of radiolabeled heterotrophic bacteria (*Vibrio natriegens*) in laboratory experiments. *V. natriegens* cells were allowed to accumulate ^{55}Fe, ^{65}Zn and ^{241}Am from seawater for 5 d and were subsequently fed to the ciliates. Uptake and depuration of the metals was monitored in the ciliates over time. Measured assimilation efficiencies were 79% for Fe, 29% for Zn and 24% for Am; corresponding efflux rates were 0.1 d^{-1} for Fe, 0.9 d^{-1} for Zn and 0.7 d^{-1} for Am. These results suggest that bacteria present an alternate source to phytoplankton for some metals (especially Fe) to be introduced into metazoan food webs.

KEY WORDS: Trophic transfer · Ciliated protozoa · Heterotrophic bacteria · Metals · Metal cycling

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INTRODUCTION

Trophic transfer studies in the marine environment most often rely on phytoplankton to represent the base of the food web under examination. The ability of microalgal cells to bioconcentrate metals of varying properties (Fisher 1986) and to transfer them to the organisms grazing on phytoplankton has been documented (Fisher et al. 1983b, 1984, Reinfelder & Fisher 1991). Few studies have examined the transfer of metals from bacterial cells to their consumers in aquatic environments (Chase & Price 1997, Maranger et al. 1998, Barbeau et al. 2001), particularly involving ciliates (Berk & Colwell 1981, Lores et al. 1999). Half of the particulate organic carbon in the world's oceans is contained in heterotrophic bacteria (Cho & Azam 1988, Fuhrman et al. 1989), illustrating their potential importance in influencing the geochemical cycling of metals and nutrients in general. The accumulation of diverse metals by bacterial cells from ambient seawater has been quantified experimentally (C. Vogel & N. S. Fisher unpubl.). Here we examined the extent to which bacterially associated metals can be transferred to protozoans that graze upon them.

Elements that are accumulated by bacteria become incorporated into the microbial loop, a highly efficient system at regenerating nutrients (Azam et al. 1983). However, the microbial loop is not a system that exists in isolation, but is also connected to the 'traditional' phytoplankton-based metazoan food chain. Therefore, bacterially bound metals may eventually enter into the larger oceanic food web. One of these links is represented by planktonic ciliates, many of which are bacterivores (Porter et al. 1985, Sherr & Sherr 1987) and ubiquitous in most aquatic systems (Beers & Stewart 1969, Fenchel 1986). Through excretion, protozoans can play a significant role in the regeneration of nutrients in the aquatic environment (Johannes 1965, Gast & Horstmann 1983, Porter et al. 1985), and ciliates in particular are suitable prey items for zooplankton such as copepods (Berk et al. 1977, Rieper 1985). Also, they may be a better dietary source for some copepods than phytoplankton based on egg development and hatching success (Heinle et al. 1977). Further, copepods assimilate certain metals (e.g. Zn) more efficiently from ciliates than from algal cells (Twining & Fisher 2004).

Different types of metals are accumulated by bacteria and some metals are bound mostly to external cell

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surfaces, while others may also move across the membranes and accumulate inside the cells. The assimilation of a metal from algal food by herbivores is tightly correlated with that metal's cytoplasmic distribution in the food (Reinfelder & Fisher 1991, 1994, Hutchins et al. 1995). A similar pattern was observed in the trophic transfer of Ag and Pb to the estuarine ciliate *Fabrea salina* feeding on the naked prymnesiophyte *Isochrysis galbana* (Fisher et al. 1995). In the present study we have chosen 3 different metals (Fe, Zn and americium [Am]) to investigate the trophic transfer to planktonic ciliates feeding on heterotrophic bacteria. Zn and particularly Fe are particle-reactive and essential trace metals that are known to be transported into bacterial cells (Tortell et al. 1996, Keung et al. 2008) and could therefore be expected to be assimilated to a relatively large extent by the ciliates. In contrast, Am is a particle-reactive element with no biological function. However, there is interest in this element from a radiological protection perspective (IAEA 2004) and its geochemical behavior is much like that of the lanthanides and some other actinides that are also particle-reactive, namely, +3 valence metals. As with algal cells (Fisher et al. 1983a), Am is expected to remain attached to the cell surfaces of bacteria and be less assimilated by the ciliates.

MATERIALS AND METHODS

Experiments were conducted in filtered natural seawater that was collected in trace-metal clean fashion from surface waters 8 km offshore from Southampton, New York. Cultures of the heterotrophic marine bacterium *Vibrio natriegens* strain Pwh3a (provided by G. Taylor) were maintained on marine agar slants (1 l seawater, 5 g bacto peptone, 1 g yeast extract) at 4°C and were subcultured periodically. In preparation for experiments *V. natriegens* was cultured in 10% marine broth (1 l seawater, 0.5 g bacto peptone, 0.1 g yeast extract) for 4 d before an inoculum was centrifuged at 1400 × *g* for 10 min and rinsed with sterile 0.2 µm filtered seawater before resuspending the cells.

Cultures of the planktonic ciliate *Uronema* sp. (provided by A. Hartz and isolated from Pacific coastal seawater in Oregon) were maintained at 17°C in sterile filtered seawater that was supplemented with a sterile rice grain and *Vibrio natriegens*, and were subcultured periodically. Before each experiment, batches of the ciliates were transferred to fresh seawater containing an inoculum of rinsed *V. natriegens*, but no rice grain, for 4 d. Preliminary experiments established that *V. natriegens* serves as an adequate food source for the ciliates. *Uronema* sp. are smaller celled, bacterivorous ciliates in the order Scuticociliatida that are abundant in coastal regions (Sherr & Sherr 1987). The average cell volume of the ciliates was 559 μm^3, which was based on the shape of a prolate ellipsoid and was calculated from length and width measurements obtained by light microscopy of 50 ind. cells.

For the trophic transfer experiments, rinsed *Vibrio natriegens* cells (10^6 cells ml^{-1}) were suspended in 800 ml of 0.2 µm filtered seawater to which radioisotope had been added 12 h before the bacterial inoculation. Radioisotopes used in these experiments included ^{55}Fe (half-life [$t_{1/2}$] = 996 d; dissolved in 0.5 N HCl) in the form of $FeCl_3$ from NEN/Perkin Elmer Life Sciences, ^{65}Zn ($t_{1/2}$ = 245 d; dissolved in 0.1 N HCl), as $ZnCl_2$ from Brookhaven National Laboratory and ^{241}Am ($t_{1/2}$ = 433 yr; dissolved in 3 N HNO_3) as $Am(NO_3)_3$ from Amersham. The trophic transfer of Fe was tested separately, whereas Zn and Am were tested simultaneously. The concentrations of the radioisotopes were 0.9 nmol l^{-1} (19.98 kBq) for ^{55}Fe, 1.94 nmol l^{-1} (39.96 kBq) for ^{65}Zn, and 1.14 nmol l^{-1} (23.68 kBq) for ^{241}Am. Each volume of seawater that received radioisotopes also first received a small amount (microliter quantities) of 0.5 N NaOH to offset the small amount (microliter quantitites) of dilute acid added along with the radioisotopes.

To determine the accumulation of the isotopes in the ciliates from bacterial food, the bacteria were exposed to radioisotopes for 5 d, after which they were collected on 0.2 µm Nuclepore polycarbonate membranes, rinsed with sterile seawater and resuspended in triplicate flasks, each containing 250 ml seawater and approximately 2 to 4 × 10^3 ciliate cells ml^{-1}. A separate set of flasks containing 250 ml seawater without ciliates also received an inoculum of radiolabeled *Vibrio natriegens* to determine the loss via desorption of the radioisotopes from bacterial cells to the water. Cellular and dissolved samples were taken periodically over a period of 4 h for Fe and 15 h for Am and Zn. At each sample time, a 1 ml unfiltered sample was taken to determine the total activity of the metal in the aqueous and the particulate phases, and a 10 ml sample was collected on 5.0 µm Nuclepore polycarbonate membranes (and rinsed 2 times with 5 ml of filtered seawater) to determine the radioactivity associated with the ciliates. In previous studies (C. Vogel & N. S. Fisher unpubl.) we found no evidence of bacterial clumping that would be caught on these filters. A 10 ml sample was also filtered (0.2 µm) and rinsed with 10 ml seawater to determine the activity of the metals associated with *V. natriegens* in the flasks containing only the bacterial cells.

To measure metal retention in ciliates, radioactive *Uronema* were removed from their radioactive bacte-

ria food and fed non-radioactive bacteria to purge any undigested bacteria and unassimilated metal. Thus, at the end of the radioactive feeding the radiolabeled ciliates that had been feeding on radiolabeled *Vibrio natriegens* were gravity filtered (5.0 µm), rinsed with seawater and resuspended into 3 flasks each containing 160 ml of seawater and 5×10^6 cells ml^{-1} of unlabeled *V. natriegens* food. Filtered (10 ml) and unfiltered (1 ml) samples were taken periodically from these flasks to determine the loss of metals from the ciliates during 28 h in the Fe experiment and 15 h in the Am and Zn experiment.

In addition, the contents of the flasks that contained only radiolabeled *Vibrio natriegens* were collected on a 0.2 µm polycarbonate filter, and the resulting radiolabeled filtrate was used to measure the accumulation of desorbed metals by unlabeled *Uronema* (added at 0.95 to 1.40×10^3 cells ml^{-1}) from the dissolved phase.

Radioactive samples were measured using a 1282 Compugamma counter (LKB Wallac) equipped with a NaI(Tl) well detector for the gamma emitting radioisotopes ^{65}Zn and ^{241}Am. Gamma emissions of ^{65}Zn were measured at 1115 keV, and at 59.5 keV for ^{241}Am. Samples containing the beta emitter ^{55}Fe were measured in a Tri-Carb 2100 TR liquid scintillation analyzer (Packard), taking a quench curve into consideration. In general, samples were counted so that propagated counting errors were <5%.

At various times throughout the experiments, samples were taken to determine the cell densities of ciliates and bacteria. *Vibrio natriegens* samples were filtered onto 0.2 µm black polycarbonate filter membranes for this purpose, and 5.0 µm filters were used for *Uronema* sp. Both types of cells were fixed with 2% final volume borate-buffered formalin and counted by 4′6-diamidino-2-phenylidole (DAPI) staining (Sherr et al. 2001) and epifluorescence microscopy using a DMB IRB inverted microscope (Leica) in all experiments.

For *Vibrio natriegens*, concentration factors for each metal were determined by dividing the radioactivity of each radioisotope in the cells by the radioactivity in the ambient seawater at the time of equilibrium with respect to metal partitioning between dissolved and particulate phases. Concentration factors were calculated on a volume/volume basis, assessed by dividing the radioactivity μm^{-3} of bacterial cells by the radioactivity μm^{-3} dissolved in the ambient seawater (Fisher et al. 1983b). The cell volume for the ciliates was calculated based on the shape of a prolate ellipsoid, with the length and width dimensions measured by means of light microscopy; the cell volume for the bacterial cells was calculated based on the shape of a rod.

Assimilation efficiencies (AE) of the metals in *Uronema* sp. were calculated based on the filtered and unfiltered samples taken during the depuration phase of the experiments. Depuration data were plotted showing the percentage of each metal retained by the ciliates upon resuspension into unlabeled seawater. Assimilation efficiencies were obtained by fitting an exponential regression to the depuration data from 2 h after the beginning of the depuration period to the last sample time. The assimilation efficiency is represented by the *y*-intercept of this curve and the slope of the curve represents the efflux rate constant (k_e) (Wang & Fisher 1999). This method of determining a metal's assimilation efficiency is appropriate for organisms for which the time required for digestion is difficult to measure. Typically, marine invertebrates, including zooplankton, display a biphasic depuration pattern with an initial rapid loss representing egestion of unassimilated material followed by a slower loss that represents loss of assimilated metal due to metabolic processes. We chose the 2 h time point as the beginning of the second phase of depuration because 2 h is a sufficiently long enough time for egestion to take place in *Uronema* sp. (Sherr et al. 1988). Essentially, the *y*-intercept of the slowly exchanging pool is equal to (the ingested metal – the egested metal)/(ingested metal).

RESULTS

The initial 5 d exposure period of *Vibrio natriegens* to the different radioisotopes resulted in volume concentration factors of 1.5×10^6 for ^{55}Fe, 8.4×10^4 for ^{65}Zn and 4.1×10^4 for ^{241}Am on the bacterial cells (Table 1). The radiolabeled *V. natriegens* retained most of the Fe that they had accumulated during the initial 5 d labeling period. After 4 h of resuspension in seawater without ciliates, the cells still retained approximately 88% of the Fe (Fig. 1). *V. natriegens* cells lost Zn to the surrounding water relatively rapidly over the course of 1 h, after which approximately 50% of this metal remained bound to the bacteria. The bacterial cells lost Zn at a slower rate from this point onward and

Table 1. Calculated volume concentration factors (VCF) in *Vibrio natriegens* during the initial 5 d labeling period, assimilation efficiencies (AE, in percent ± SE) of the 3 metals in *Uronema* sp., daily efflux rates (k_e) of the metals from the ciliates, and concentrations of the 3 metals μm^{-3} in each of the 2 study organisms at the end of the metal uptake phase (bacterial cells and ciliates)

	Bacterial VCF	Ciliate AE (%)	Ciliate k_e (d^{-1})	amol μm^{-3} bacteria	amol μm^{-3} ciliate
Fe	1.50×10^6	79 ± 3	0.1	0.55	0.17
Zn	8.43×10^4	29 ± 2	0.9	0.14	0.07
Am	4.14×10^4	24 ± 1	0.7	0.04	0.02

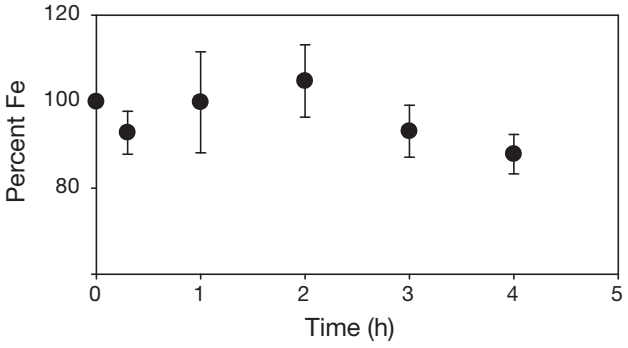

Fig. 1. Percentage of ^{55}Fe retained by *Vibrio natriegens* over time after resuspending the cells into sterile filtered, unlabeled seawater. Data are means (± SE) of 3 replicates

retained 30% of this metal by the final sample time. Am was also lost relatively rapidly from the bacteria over the first 1 h, at which time 40% of it remained associated with bacterial cells. After 1 h, Am also desorbed from the cells at a slower rate, until 23% of Am remained cell-bound at the final sample time (Fig. 2).

Once the radiolabeled bacteria were available for consumption by the ciliates, approximately 65% of the Fe bound to bacterial cells was transferred to the ciliates due to ingestion of the bacteria (Fig. 3). Of the ^{55}Fe found in the ciliates, up to 15% may have been taken up from the aqueous phase resulting from desorption from bacteria and the remainder from ingestion of the radiolabeled bacteria. The initial dietary uptake of Fe by *Uronema* sp. occurred rapidly and leveled off after 1 h. At the end of the dietary uptake phase, the radioactivity associated with the ciliates had decreased slightly (to 52%), indicating a release due to cycling of the metal. Upon ingestion of *Vibrio natriegens*, roughly 20% of the bacterially bound Zn was transferred to the ciliates, and approximately 20 to 25% of Am (Fig. 4). For Zn, uptake of desorbed metal may have accounted for up to 9%, and for Am up to 8% (Fig. 4). The assimilation efficiencies of the 3 metals in *Uronema* sp. from ingested bacteria were about 79% for Fe, 29% for Zn and 24% for Am (Table 1). The mean concentration of each metal μm^{-3} of cell was determined for *V. natriegens* and *Uronema* sp. cells using the specific activity of each radioisotope. Concentrations in bacterial cells were 2 to 3 times those in the ciliates (Table 1).

At the end of the depuration period, a mean value of 75.4% of the Fe that the *Uronema* sp. had accumulated by feeding on *Vibrio natriegens* remained in or bound to the ciliates (Fig. 5), whereas only about 20% of the Zn and Am remained associated with the ciliates (Fig. 6). Depuration occurred in 2 phases; an initial phase characterized by a faster loss rate of the metal lasting about 1 h, followed by a slower loss phase. Efflux rates (d^{-1}) of assimilated metals from *Uronema* sp. were only calculated for the second, slower loss phase and were 0.1 for Fe, 0.9 for Zn and 0.7 for Am (Table 1). Efflux rate constants were calculated for the slower (second) loss phase only as this phase represents metal loss due to metabolic activities.

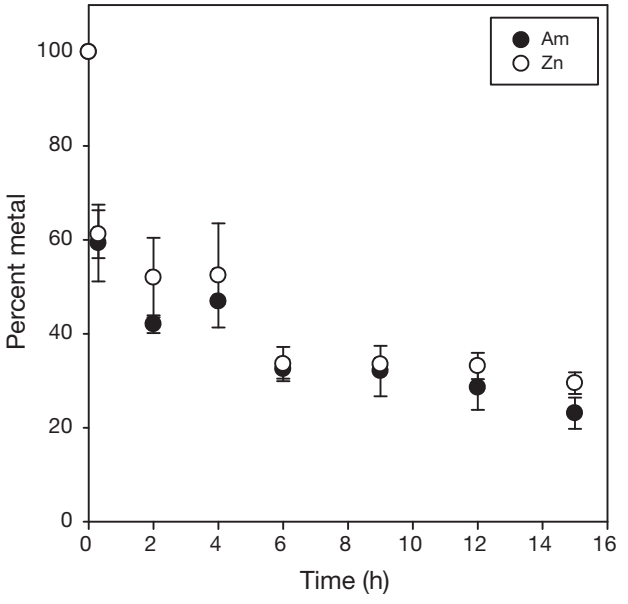

Fig. 2. Percentage of ^{241}Am and ^{65}Zn retained by *Vibrio natriegens* over time after resuspending the cells into sterile filtered, unlabeled seawater. Data are means (± SE) of 3 replicates

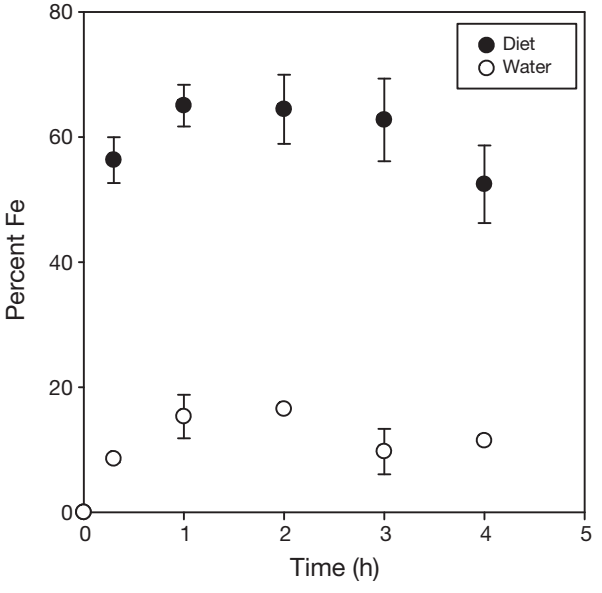

Fig. 3. Percentage of total ^{55}Fe in the culture associated with *Uronema* sp. over time while ingesting radiolabeled *Vibrio natriegens* (i.e. 'diet'), and accumulation of ^{55}Fe by *Uronema* sp. desorbed from *V. natriegens* cells (i.e. 'water'). Data are means (± SE) of 3 replicates

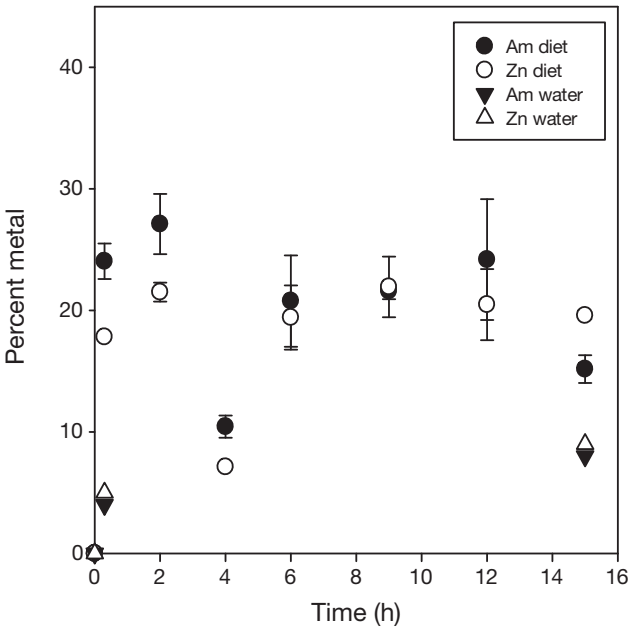

Fig. 4. Percentage of total ^{241}Am and ^{65}Zn in the culture associated with *Uronema* sp. over time while ingesting radiolabeled *Vibrio natriegens* (i.e. 'Am diet', 'Zn diet'), and accumulation of ^{241}Am and ^{65}Zm by *Uronema* sp. desorbed from *V. natriegens* cells (i.e. 'Am water', 'Zn water'). Data are means (± SE) of 3 replicates for dietary uptake

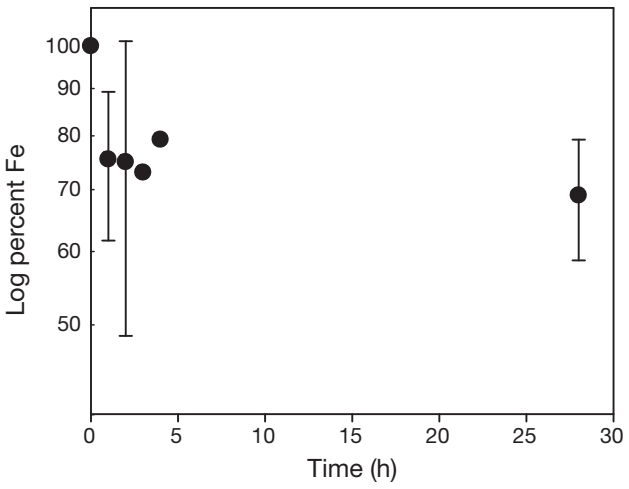

Fig. 5. Percentage of ^{55}Fe retained by *Uronema* sp. over time during the depuration period in unlabeled seawater. Data are means (± SE) of 2 replicates

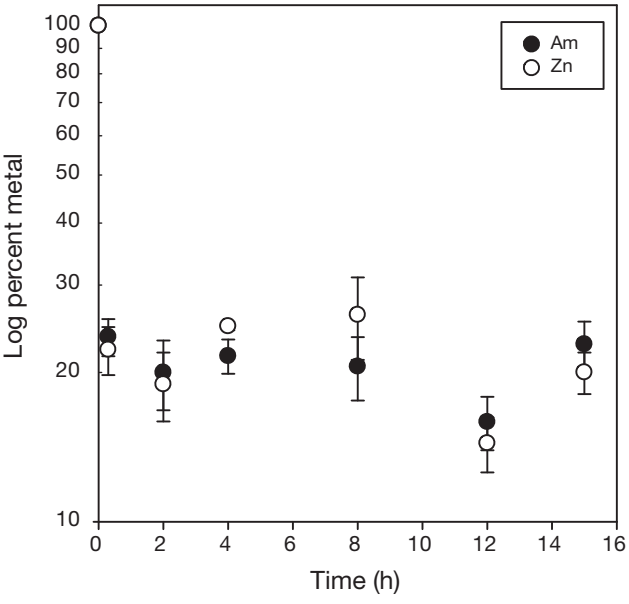

Fig. 6. Percentage of ^{241}Am and ^{65}Zn retained by *Uronema* sp. over time during the depuration period in unlabeled seawater. Data are means (± SE) of 3 replicates

DISCUSSION

Ciliated protozoa have been noted for their potential to be used as indicator organisms to assess the pollution of sediments and water in a variety of systems (Slabbing et al. 1990, Foissner 1999), including the monitoring of toxic effects of heavy metals in sewage sludge (Madoni et al. 1996, Martin-Gonzalez et al. 2005). In addition to their role as bioindicator organisms, ciliates are abundant and ubiquitous in benthic and pelagic environments where they can serve as a link between microbial loop organisms and animals in the metazoan food chain. Ciliates provide copepod predators with similar amounts of C as phytoplankton cells (Rieper 1985) and may even increase their egg hatching success (Heinle et al. 1977). They exhibit growth efficiencies of up to 40% when consuming bacterial cells (Ducklow 1983), indicating a relatively efficient transfer of energy and nutrients between the 2 trophic levels.

Uronema sp. can ingest about 700 bacterial cells h^{-1}, and the process of digestion occurs within about 25 min (Sherr et al. 1988), which explains the sharp increase in the percentage of all 3 metals found associated with the ciliates within only 1 h of feeding on the radiolabeled *Vibrio natriegens*. The fluctuation of radioactivity in the ciliates between sample times during the dietary uptake (seen for Am and Zn) can probably be attributed to cycling of these elements through the cells. Individual protozoan digestive vacuoles have a life span of 20 to 60 min from the time of formation to the point where their remaining contents are expelled (Fok et al. 1982). If ingestion and digestion occurs within such short timescales, then it can be expected that the ciliates in the present experiments were repeatedly ingesting bacterial cells and excreting waste products over the course of the dietary uptake phase.

When assessing assimilation efficiencies in higher (multicellular) animals, gut passage time is often taken into account as a factor that can have an influence on this process (Wang & Fisher 1999). This is based on the fact that organisms with longer gut passage times generally display high assimilation of contaminants due to the extended time period the digestive enzymes have to act on the food items (Sibly & Calow 1986). Ciliates, on the other hand, produce food vacuoles in which prey items are contained and processed, a process that usually occurs on shorter timescales than it does for higher animals. The microenvironment of the food vacuoles is acidic compared with the ciliates' external environment. A pH range between 2 and 4 during active digestion (Howland 1928, Fok et al. 1982) may not only serve to kill ingested cells (Fok et al. 1982), but also create a tendency for metals to occur in soluble form (Millero 1998), and possibly to be transported into the ciliate cytoplasm rather than being egested or excreted. This is of interest especially for a metal such as Fe, which is not very soluble in seawater and readily forms colloidal precipitates or oxides that display reduced bioavailability for microorganisms (Rich & Morel 1990). However, the acidity of the food vacuole leads to dissolution of this colloidal Fe and, therefore, an increase of its bioavailable form (Barbeau et al. 1996, Barbeau & Moffett 1998). Once these complexes have dissolved, it is possible that the now soluble metal is transported across the vacuole membrane and into the cytoplasm of the cell leading to its accumulation inside the organism. It is possible that these conditions account, at least partially, for the high assimilation efficiency of Fe (79%) that was measured in the present study. In addition, Fe is mostly stored inside the cells, and therefore less likely to be excreted by the organisms. Since it is required as an enzyme cofactor for a variety of metabolic reactions, Fe should be retained by the cells rather efficiently as was illustrated by its low efflux rate from the ciliates.

The assimilation efficiency of Fe in the ciliates in the present study was higher than the 36 and 25% we calculated from Chase & Price's (1997) study in the microflagellate *Paraphysomonas imperforata* under high and low Fe exposures, respectively. They found that 58 to 75% of the ingested Fe was excreted, whereas the ciliates in our study retained 75% of the Fe at the end of the depuration period. Chase & Price (1997) also exposed their bacterial food to greater Fe concentrations than we used (0.9 nM), i.e. 12.5 nM (low Fe treatment) and 8.4 µM (high Fe treatment). Thus, the flagellates in their study ingested substantially greater amounts of Fe than did the ciliates in our study and, together with physiological differences between the 2 protozoans, this may contribute to the differences between the 2 sets of assimilation efficiencies.

Of the 3 metals analyzed, Fe is also accumulated by heterotrophic marine bacteria to the greatest extent (volume concentration factor [VCF] of 1.5×10^6 in the present study). It can be assumed that at least 20% of the bioavailable Fe in surface ocean waters is bound to bacterial cells, and that the majority of it is located inside the cells (C. Vogel & N. S. Fisher unpubl.). Given the high assimilation efficiency of Fe in *Uronema* sp. in the present study, a significant portion of the bacterially bound Fe is transferred to planktonic ciliates, which may in turn be consumed by zooplankton and then throughout the food web. The relatively low efflux rate of Fe from ciliates (k_e = 0.1 d^{-1}) further strengthens the potential of Fe transfer to the next trophic level. Copepods have displayed an Fe assimilation efficiency of 32% when their food consists of ciliates (Twining & Fisher 2004). The assimilation efficiency of Am in ciliates feeding on bacteria was the lowest of the 3 metals analyzed, as might be expected given that Am is a nonessential metal that mainly remains bound to cell surfaces (Fisher et al. 1983a). When bacteria are exposed to Zn over a range of ambient concentrations, less than half of the cellular Zn is transported into the cytoplasm (Keung et al. 2008), which is consistent with the assimilation efficiency observed for Zn in the ciliates.

We can compare the assimilation efficiencies of metals ingested by ciliates from bacteria to those in copepods feeding on ciliates and phytoplankton cells. Copepods feeding on ciliates assimilated about 32% of Fe and 77% of Zn (Twining & Fisher 2004). Copepods feeding on log-phase diatoms assimilated about 5 to 16% of ingested Fe (Hutchins et al. 1995), 27% of Zn and 1% of Am (Reinfelder & Fisher 1991). The observed assimilation of Fe is greater in ciliates than in copepods and may be due to physiological differences or requirements between protozoans and crustaceans. Differences in the pH of the digestive organs in ciliates and copepods may also account for these differences. Actively feeding copepods have a gut pH >7 (Pond et al. 1995), which is significantly higher than that reported by Howland (1928) and Fok et al. (1982) for ciliate digestive vacuoles (pH 2 to 4). As noted above, the higher acidity of the ciliate vacuoles may explain the higher assimilation efficiency for Fe observed in ciliates than that seen in their predators.

While metals can be taken up by ciliates from the aqueous phase and from diet, our results indicate that the latter predominates. Thus, metals that are assimilated by a predator have the greatest potential to be transferred to the next higher trophic level. Generally, ingested material that is not assimilated is either egested in the form of discrete fecal pellets (e.g. in copepods), or excreted in dissolved form or egested as small amorphous particles, as is the case for ciliates

(Elliott & Clemmons 1966, Stoecker 1984). The fecal material produced by ciliates is not densely packaged and tends to break apart into fine particulate matter upon egestion (Elliott & Clemmons 1966, Sieburth et al. 1978). Therefore, it is unlikely that metals incorporated in these small particles will enter the particulate flux of organic matter that sinks out of the photic zone, as is the case for copepod fecal pellets. Instead, metals not assimilated by ciliates are most probably recycled in surface waters where they, like excreted nutrients, and can become available again for accumulation by other planktonic organisms, including bacteria (Prast et al. 2007) and phytoplankton (Ota & Taniguchi 2003). Finally, the lower concentration of metals observed in ciliates compared to that seen in their bacterial food is consistent with the general observation that most metals do not display biomagnification in marine food chains, methylmercury and cesium being notable exceptions (IAEA 2004, Mathews & Fisher 2008).

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