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Bioaccumulation, Subcellular Distribution, and Trophic Transfer of Copper in a Coastal Marine Diatom

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Cellular uptake and subcellular distribution of copper were measured in the coastal marine diatom *Thalassiosira weissflogii* grown with free Cu ion concentrations ($10^{-14.79}$—$10^{-9.79}$ M) that are typical of coastal waters. Intracellular Cu quotas increased 2−3-fold and 4−5-fold per decade increase in free Cu ion concentration over the pCu (pCu = −log [Cu^{2+}]) ranges of 14.79−11.79 and 11.79−9.79, respectively. The trophically available cytoplasmic pool of Cu increased by 2 orders of magnitude in *T. weissflogii* cells (0.53×10^{-17}−75×10^{-17} mol Cu $cell^{-1}$) grown with $10^{-14.8}$−$10^{-9.8}$ M free Cu. A 2-fold increase in intracellular and cytoplasmic Cu concentrations was observed in response to lowering the free concentration of the potentially antagonistic metal Zn by a factor of 10. However, the percent of intracellular Cu in the cytoplasmic fraction of *T. weissflogii* (≅ 40%) was relatively constant over the range of cupric ion activities and [Cu^{2+}]:[Zn^{2+}] ratios applied. The assimilation efficiency (AE) of copper in marine copepods fed *T. weissflogii*, determined using the inert-tracer ratio method, was 40.3 ± 6.3% for copepods fed diatoms grown at pCu = 12.79 and 50.3 ± 9.7% for copepods fed diatoms grown at pCu = 10.79. Our results suggest that, above free Cu concentrations of $10^{-11.8}$ M, the availability of Cu for trophic transfer in coastal waters increases as a result of rapidly increasing concentrations of Cu in the assimilable, cytoplasmic fraction of diatoms and potentially higher Cu AEs at the base of the pelagic food web.

Introduction

Copper is a potentially toxic trace metal and like Cd, Pb, and Zn is present at elevated concentrations in coastal waters due to anthropogenic inputs (*1, 2*). Recently, considerable efforts have been made to study the chemical speciation of copper in natural waters because copper can be highly toxic to aquatic organisms as well as a micronutrient in some species (*3*−*5*). With a few exceptions (*6, 7*), the biological availability of Cu is proportional to the concentration of the free copper ion, as is the case for other biologically active metals such as Zn and Cd (*8, 9*). Complexation by organic ligands maintains free Cu ion concentrations as low as $10^{-13.5}$ M in coastal and oceanic surface waters (*3, 10*−*12*), but small increases in total dissolved Cu can titrate copper-complexing organic ligands, resulting in large increases in free Cu ion concentration (*2, 4*). Several studies show that free Cu concentrations in the range of 10^{-12}−10^{-9} M can cause growth inhibition and metabolic stress in estuarine phytoplankton (*13*−*15*), cyanobacteria (*16*), and zooplankton (*17*). However, there are few data that can be used to evaluate directly the impact of variable free Cu ion concentrations on the bioaccumulation of copper in such organisms and trophic transfer to consumers.

The accumulation and intracellular distributions of metals in phytoplankton affect the trophic transfer of metals in aquatic herbivores as well as metal remineralization rates in sinking phytodetritus. The soluble fraction of metal associated with the cytoplasm of phytoplankton cells is assimilated by herbivorous consumers such as copepods and bivalve larvae (*18*−*22*), while metals bound to phytoplankton cell membranes are not assimilated and are egested in fecal material. Similarly, metals associated with the cytoplasm of phytoplankton cells are recycled rapidly in surface waters from decaying phytoplankton debris, but membrane-bound metals are remineralized more slowly in the upper water column (*23*).

A variety of biologically active (Cd, Co, Fe, Se, and Zn) and nonactive (Ag, Am, and Hg) trace elements have been extensively studied with respect to their cellular distribution in phytoplankton and assimilation efficiency in aquatic animals using appropriate radiotracers. Despite its importance as a contaminant in natural waters and a micronutrient in aquatic organisms, copper has been relatively little studied with respect to phytoplankton uptake and trophic transfer due to the lack of a long-lived Cu radioisotope or a stable isotope of low mass abundance.

In this work, we studied the accumulation and subcellular distribution of copper in the coastal diatom *Thalassiosira weissflogii* as a function of free Cu ion concentrations. Cu exhibits accumulation antagonisms with Zn and other metals in marine plankton (*24*). We therefore studied the effect of Zn on Cu uptake and subcellular distribution. We also examined the efficiency of copper assimilation in copepods fed diatoms acclimated to two free Cu concentrations.

Materials and Methods

Copper Uptake by Diatoms. Copper accumulation experiments were conducted with the coastal diatom *T. weissflogii* (clone ACTIN). Axenic cultures were maintained in Aquil medium (*25*) with added macronutrients (N, P, and Si), vitamins, trace metals, and EDTA under continuous (24 h) illumination (200 μmol quanta m^{-2} s^{-1} PAR) at 18 °C. Copper was added as a 1:1 (mol:mol) Cu:EDTA solution so that its addition, especially at higher concentrations, did not change the free ion activities of other trace metals. Free ion activities of copper and other trace metals in the growth media were calculated using the MINEQL speciation program (*26*). Experimental media had free Cu ion activities of $10^{-14.79}$—$10^{-9.79}$ M, which are typical of coastal surface waters (*12*). After the addition of trace metals and Cu-EDTA stock solutions, the experimental media were equilibrated for 24 h before inoculation. Cells were transferred from stock culture tubes to experimental media at cell densities of 200−300 cells mL^{-1}. Culture growth was monitored by measuring in vivo chlorophyll-a fluorescence (*27*) and cells were counted by microscope. Cells were grown for 3−4 days (6−8 generations) and harvested during exponential growth.

Cellular Cu concentrations were determined by graphite furnace atomic absorption spectrometry (GFAAS) after acid digestion (*28*). Cellular Cu concentrations were converted to molar Cu:C ratios using an average C:cell of 13.0 ± 0.70 pmol for *T. weissflogii* grown at pCu = 13.79. Cellular organic carbon was measured in cells collected on precombusted glass fiber filters (GF/F) with a Carlo-Erba CHN analyzer.

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Subcellular Fractionation of Copper. Subcellular partitioning of copper in the cells was determined by measuring copper concentrations in extracellular (surface-bound), cytoplasmic, and membrane-bound fractions (*18, 29*). Cell fractionation of Cu was measured in exponential and stationary growth phase cells from the same Cu accumulation cultures described above. Briefly, a 50 mL subsample was filtered through a 3 μm polycarbonate (PC) membrane filter under low vacuum pressure (<130 mmHg) to minimize cell breakage. To remove surface-bound Cu, cells were sequentially rinsed with 10 mL of 1 mM EDTA in ion-exchange resin (Chelex)-treated (to remove trace metals) synthetic ocean water (SOW) followed by 10 mL of trace metal-free SOW. The filter with cells was transferred to a 1.25 mL acid-cleaned centrifugation tube and 1 mL of trace metal-free SOW was added. After the filter was removed, cells were homogenized in an ice bath (<5 °C) by sonification (Fisher, Model 60) with two sets of 20 1−2 s pulses at 6−7 W, and this homogenized sample was centrifuged at 14 000*g* for 4 min at room temperature. The supernatant was decanted, and the pellet was washed with 1 mL of trace metal-free SOW. Copper in the pellet was then determined by GFAAS after acid digestion. Cytoplasmic Cu concentrations were calculated as the difference between intracellular Cu (EDTA-washed cells) and Cu in the membrane fraction (pellet). The membrane fraction contains plasma membranes, organelles (e.g. chloroplasts, mitochondria), and the diatom cell wall. Copper concentrations associated with cell surfaces were estimated from cellular Cu concentrations before and after EDTA washing. The effect of Zn on cellular Cu quotas and intracellular distributions was determined by comparing results with *T. weissflogii* cells grown in Zn-sufficient (pZn = 10.88) and Zn-limiting AQUIL medium (pZn = 11.58 and 11.88). The cellular fractionation of Cu was determined in three subsamples per culture from each of three to six replicate cultures.

Assimilation of Copper by Copepods. Copepods of the genera *Acartia* (90% of experimental animals by number) and *Temora* (10%) were collected with a plankton net (63 μm Nylon mesh) from Cheesequake Creek near Raritan Bay, NJ in summer. Adult copepods were separated from nauplii and maintained in the laboratory in glass-fiber-filtered Cheesequake Creek water (S = 24‰) at 18 °C and fed the diatom *T. weissflogii*. Adult copepods were transferred to SOW and acclimated for 2 or 3 days prior to feeding experiments. Feeding experiments were performed using *T. weissflogii* grown at pCu 12.79 or 10.79. Cells in late exponential growth were collected on 3 μm PC membrane filters and washed sequentially with 1 mM EDTA and SOW to remove surface-bound copper. Cells were resuspended in SOW to give cell concentrations in the experimental feeding suspensions ranging from 7×10^4 to 1×10^5 cells mL^{-1}. After a 1 h preincubation of the cells in SOW, copepods were added to give an average copepod density of 0.5−1.0 ind. mL^{-1}. After 12−24 h feeding on diatoms in the dark at 18 °C, copepods were removed with a 160 μm nylon mesh from the feeding suspension. Fecal pellets produced by the copepods during the feeding period were collected on a 40 μm nylon mesh, rinsed with 10 mL SOW, and resuspended in 20 mL of SOW. The feces resuspension solution was divided for separate determinations of Si (4 mL) and Cu (16 mL), and fecal pellets were collected on 3 μm PC membrane filters. The assimilation efficiency (AE) of copper in diatom-fed copepods was determined by the inert tracer ratio method using the silicon of the diatom frustule as the unassimilated tracer (*30*). In this method, AE is calculated as

$$\mathrm{AE} = [1 - (\mathrm{Cu/Si})_{\mathrm{feces}}/(\mathrm{Cu/Si})_{\mathrm{food}}] \times 100 \qquad (1)$$

where AE is the assimilation efficiency (%), $(\mathrm{Cu/Si})_{\mathrm{feces}}$ is the ratio of Cu to Si in copepod fecal pellets, and $(\mathrm{Cu/Si})_{\mathrm{food}}$ is the ratio of Cu to Si in the diatoms. The Cu and Si content of *T. weissflogii* cells were determined at the start of each feeding experiment. To determine the loss of Cu and Si from fecal pellets during the feeding period, fecal pellets collected from animals fed for 12−24 h were incubated in 30 mL of SOW at room temperature for 24 h. Periodically, a 5 mL aliquot was pipetted from the resuspension solution after gentle shaking and filtered onto a 3 μm PC membrane filter, and the copper and silicon contents were measured.

TABLE 1. Cellular Distributions of Copper in *T. weissflogii*[a] Grown in a Range of Free Copper Ion Concentrations (pCu = − log [Cu^{2+}]) and Molar Ratios of Cellular Copper to Carbon (Cu:C)

	Cu concentrations in *T. weissflogii* (10^{-17} mol cell^{-1})			
pCu	cytoplasmic	membrane-bound	surface-bound	Cu:C[b] (μmol:mol)
		Exponential Growth Phase		
14.79	0.53 ± 0.01	0.86 ± 0.07	0.08 ± 0.06	1.07
13.79	0.96 ± 0.14	1.47 ± 0.08	0.11 ± 0.08	1.87
12.79	2.21 ± 0.34	2.85 ± 1.42	0.79 ± 0.06	3.89
11.79	3.18 ± 0.24	5.53 ± 1.15	2.37 ± 0.25	6.70
10.79	13.30 ± 2.78	25.49 ± 3.16	14.24 ± 0.73	29.80
9.79	74.96 ± 11.62	127.40 ± 8.26	50.82 ± 3.23	155.66
		Stationary Growth Phase		
13.79	1.25 ± 0.06	1.61 ± 0.04	0.13 ± 0.01	2.20
11.79	18.39 ± 3.13	18.25 ± 2.81	9.52 ± 0.81	28.18

[a] Means and standard deviations for exponential phase and stationary phase cultures (n = 2−4) are shown. [b] Estimation of cellular Cu:C excluded the surface-bound fraction of copper in the cells.

Analyses of Cu and Si in Diatom Cells and Fecal Pellets. For copper measurement, PC filters with collected diatom cells or fecal pellets were placed in a 30 mL fused-quartz crucible, and 4 mL of concentrated nitric acid (Optima grade, Fisher) was added. After 24 h incubation, filters were removed, and the solution was slowly evaporated by gentle heating (90 °C). The residue was redissolved in 1 mL of 1% nitric acid, and copper concentration was measured by GFAAS (Perkin-Elmer 4100ZL) using an external calibration method or a standard addition method. Quality assurance samples (NIST SRM 1643d, "trace elements in water") were analyzed for each set of samples, and cellular Cu concentrations were corrected for method blanks of 0.12 ± 0.05 μg Cu L^{-1} in the final extract. Determination of the silicon content in diatom cells and fecal pellets followed the hydrolysis-colorimetric method (*31*). Briefly, 10 mL of diatom cell suspension from each feeding experiment was filtered onto a 3 μm PC membrane filter. Collected diatom cells and fecal pellets were resuspended into 3 mL of 0.5% (w/v) Na_2CO_3 solution. After heating for 1 h at 85 °C, the solution was cooled to room temperature, and the pH of the solution was adjusted to 3−4 with 0.5 N HCl (Optima, Fisher). The solution was centrifuged, and 1 mL of supernatant was taken for the colorimetric measurement of silicic acid (*30*).

Results and Discussion

Accumulation and Subcellular Distribution of Cu in the Diatom, *T. weissflogii*. Copper accumulation in *T. weissflogii* was quantified as intracellular (cytoplasmic plus strongly membrane-bound) and surface-bound (EDTA-removable) Cu quotas (mol cell^{-1}, Table 1). A positive relationship between intracellular Cu quotas and cupric ion activities was evident from the Cu uptake results over a broad range of free Cu concentrations ($10^{-14.79}$−$10^{-9.79}$; Figure 1). Cell growth rates ranged from 1.2 to 1.4 d^{-1} at all experimental cupric ion activities, consistent with the lack of growth inhibition by Cu in nutrient replete media at these concentrations (*15*). There was no saturation observed for copper accumulation by *T.*

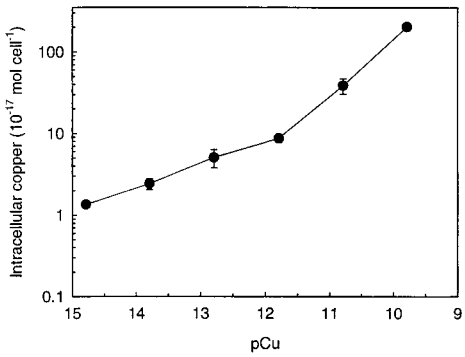

FIGURE 1. Intracellular (total minus surface adsorbed) Cu concentrations in *T. weissflogii* as a function of free copper ion concentration (pCu = −log [Cu^{2+}]). Data points are means of three to six replicates with 1 SD error bars. For 14.79 > pCu > 11.79, log $Cu_{intracell}$ = 0.271 ± 0.0093 log [Cu^{2+}] −12.9 ± 0.12 (r^2 = 0.997) and for 11.79 > pCu > 9.79, log $Cu_{intracell}$ = 0.685 ± 0.020 log [Cu^{2+}] −8.00 ± 0.22 (r^2 = 0.999), where $Cu_{intracell}$ = mol Cu cell^{-1}.

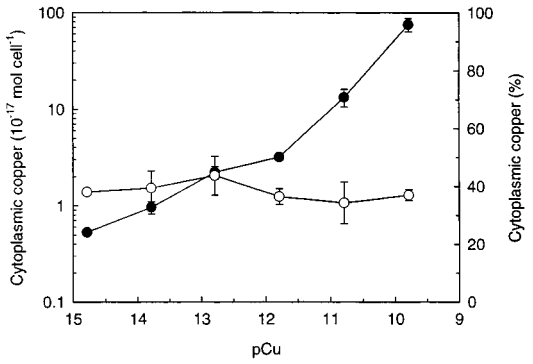

FIGURE 2. Concentration (●) and percent (○) of intracellular Cu in the cytoplasm of the diatom, *T. weissflogii* as a function of free copper ion concentration (pCu = −log [Cu^{2+}]). Data points are means of three to six replicates with 1 SD error bars. For 14.79 > pCu > 11.79, log Cu_{cyto} = 0.270 ± 0.026 log [Cu^{2+}] −12.9 ± 0.12 (r^2 = 0.981) and for 11.79 > pCu > 9.79, log Cu_{cyto} = 0.685 ± 0.038 log [Cu^{2+}] −8.45 ± 0.41 (r^2 = 0.997), where Cu_{cyto} = mol Cu cell^{-1}.

weissflogii up to the highest cupric ion activity of $10^{-9.79}$ M. Our data clearly indicate that the cellular levels of Cu in *T. weissflogii* are primarily controlled by free Cu ion activities in the growth medium consistent with the "free ion activity model" of metal bioavailability (*7, 8*). A similar pattern of cellular Cu uptake was reported for a number of phytoplankton species by Sunda and Huntsman (*5*) who found a near-linear relationship between intracellular Cu and free Cu ion concentrations over a pCu range of 15.12−10.52. In contrast with Sunda and Huntsman, however, we found that *T. weissflogii* contains measurable intracellular Cu (14−24 amol cell^{-1}) when grown in media with low free Cu concentrations (pCu 14.79−13.79; Table 1). Experimental differences, including differences in light (Sunda and Huntsman used 500 μmol quanta m^{-2} s^{-1} PAR on a 14:10 L/D light cycle compared with continuous illumination at 200 μmol quanta m^{-2} s^{-1} PAR used in our study) and growth rates ($\mu \sim 1.2$ d^{-1} for our cultures, $\mu \sim 0.6$ d^{-1} in Sunda and Huntsman (5)) may account for this discrepancy. These differences may have contributed to a greater biological demand for Cu or greater Cu uptake via Zn or Mn transport systems in our *T. weissflogii* cultures. Experimental differences may also have affected the chemistry of the culture medium and, as a result, Cu bioavailability.

Inracellular Cu concentrations in exponential growth phase cells increased only 2−3-fold with a 10-fold increase in free Cu ion concentration over the pCu range of 14.79−11.79 but increased by 4−5-fold per order of magnitude increase in Cu^{2+} concentration from pCu 11.79 to 9.79 (Figure 1). Similar biphasic patterns of Cu uptake were found in a freshwater green alga (*32*) and a number of marine phytoplankters (*5*). Knauer et al. (*32*) suggested that Cu uptake by a freshwater green alga *Scendesmus subspicatus* is mediated by two systems: one with high affinity that operates over the pCu range 14−12 and a low affinity system that operates at [Cu^{2+}] > 10^{-12} M. It is interesting to note that Cu quotas in *T. weissflogii* begin to increase rapidly at a pCu of 11.79, similar to the pCu at which Knauer et al. (*32*) observed a switch in Cu accumulation in *S. subspicatus*. If *T. weissflogii* does indeed activate a low affinity Cu uptake system above [Cu^{2+}] of $10^{-11.79}$ M, doing so is only partially effective in regulating intracelluluar Cu.

The Cu contents of our laboratory diatom cultures can be compared with those of natural phytoplankton assemblages using estimated Cu:C ratios. Based on our organic carbon contents in cells grown with pCu = 13.79 and whole cell Cu concentrations, cellular Cu:C ratios in *T. weissflogii* ranged from 1.1 to 6.7 μmol mol^{-1} over free Cu ion concentrations ranging from $10^{-14.79}$ to $10^{-11.79}$, where Cu:C ratios increased linearly by about a factor of 2 with a 10-fold increase in free Cu ion concentration (Table 1). There are few data on the Cu:C ratios of phytoplankton in field samples from coastal waters. However, the Cu:C ratios we estimated for *T. weissflogii* grown at free Cu ion concentrations of $10^{-14.79}$−$10^{-12.79}$ M are comparable with average Cu:C values of 2.8−4.1 μmol mol^{-1} found in natural plankton samples for North Atlantic and North Pacific waters (*5, 33, 34*) where free Cu ion concentrations range from $10^{-13.5}$ to $10^{-12.7}$ (*11*).

TABLE 2. Intracellular Concentrations (Total Minus Surface Bound) and Cytoplasmic Fractions of Copper and Specific Growth Rates (μ) in *T. weissflogii* Grown at Six [Cu^{2+}]:[Zn^{2+}] Ratios (pM = −log [M^{2+}])[a]

pCu	pZn	$[Cu]_{cell}$ (10^{-17} mol cell^{-1})	$Cu_{cytoplasm}$ (%)	μ (d^{-1})
13.79	10.88	2.40 ± 0.37	40.9 ± 4.3	1.20 ± 0.09
	11.58	2.47 ± 0.08	35.7 ± 2.3	0.97 ± 0.01
	11.88	4.29 ± 0.37	37.4 ± 7.1	0.43 ± 0.03
11.79	10.88	8.71 ± 0.91	40.5 ± 7.6	1.19 ± 0.10
	11.58	9.01 ± 0.50	35.8 ± 5.1	1.03 ± 0.10
	11.88	19.63 ± 2.28	40.8 ± 6.4	0.40 ± 0.04

[a] Cu data and growth rates are means and standard deviations of three replicate samples from each of the six cultures.

The proportion of total cellular Cu bound to the cell surface (EDTA-removable) of *T. weissflogii* was ~5% at pCu = 14.79 and increased to 25% at higher free Cu ion concentrations (pCu = 10.79−9.79; Table 1). The cytoplasmic Cu content of *T. weissflogii* cells increased from 0.53 × 10^{-17} to 75 × 10^{-17} mol Cu cell^{-1} in cells grown with $10^{-14.8}$−$10^{-9.8}$ M free Cu ion and, like total intracellular Cu, showed a biphasic pattern (Figure 2). The concentration of cytoplasmic Cu increased 2.5 times faster with increasing [Cu^{2+}] at concentrations greater than $10^{-11.79}$ M than at lower concentrations (Figure 2). The percent of intracellular (total minus surface bound) Cu in the cytoplasm of *T. weissflogii* (38.2 ± 3.2%), however, was relatively constant over the range of cupric ion activities applied (Figure 2). Similarly, while intracellular and cytoplasmic Cu concentrations in *T. weissflogii* increased 2-fold in highly Zn-limited cells (pZn = 11.88; Table 2), cell fractionation of Zn-limited cultures showed an average of 38.5 ± 2.5% of cellular copper in the cytoplasm and no difference in the distributions of subcellular Cu between Zn-sufficient and Zn-limited cultures. Thus, the antagonistic interaction between Cu and Zn (*35*) affects total Cu uptake but not cellular fractionation. Joux-Arab et al. (*36*) found 37% of Cu in the pennate diatom *Haslea ostrearia* in the

TABLE 3. Concentrations of Copper and Silicon in Ingested and Egested Materials and Assimilation Efficiencies of Copper in Copepods Based on the Ratio Method

	concentration in food (mol cell^{-1})			concentration in fecal pellets (mol)[a]			assimilation
[Cu^{2+}]	Cu	Si	$(Cu/Si)_{food}$	Cu	Si	$(Cu/Si)_{feces}$	efficiency (%)[b]
$10^{-12.8}$	5.75×10^{-17}	5.45×10^{-13}	1.06×10^{-4}	1.24×10^{-11}	1.85×10^{-7}	6.70×10^{-5}	36.8
	5.75×10^{-17}	5.45×10^{-13}	1.06×10^{-4}	2.08×10^{-11}	3.74×10^{-7}	5.56×10^{-5}	47.5
	5.75×10^{-17}	5.45×10^{-13}	1.06×10^{-4}	2.41×10^{-12}	3.58×10^{-8}	6.73×10^{-5}	36.5
$10^{-10.8}$	3.51×10^{-16}	6.32×10^{-13}	5.55×10^{-4}	1.15×10^{-10}	4.69×10^{-7}	2.45×10^{-4}	55.9
	3.93×10^{-16}	6.62×10^{-13}	5.94×10^{-4}	1.15×10^{-10}	5.02×10^{-7}	2.29×10^{-4}	61.4
	4.24×10^{-16}	6.48×10^{-13}	6.54×10^{-4}	8.21×10^{-11}	2.78×10^{-7}	2.95×10^{-4}	54.9
	4.29×10^{-16}	6.64×10^{-13}	6.46×10^{-4}	7.29×10^{-11}	1.61×10^{-7}	4.53×10^{-4}	29.9
	6.37×10^{-16}	5.64×10^{-13}	1.13×10^{-3}	8.66×10^{-11}	1.75×10^{-7}	4.95×10^{-4}	56.2
	6.37×10^{-16}	5.64×10^{-13}	1.13×10^{-3}	8.03×10^{-10}	1.30×10^{-6}	6.18×10^{-4}	45.3
	6.37×10^{-16}	5.64×10^{-13}	1.13×10^{-3}	1.01×10^{-10}	1.72×10^{-7}	5.87×10^{-4}	48.1
	2.04×10^{-15}	5.41×10^{-13}	3.77×10^{-3}	4.63×10^{-10}	2.48×10^{-7}	1.87×10^{-3}	50.4

[a] Concentrations of copper and silicon in fecal pellets collected in each feeding experiment. [b] Separate batches of copepods were used in each AE experiment, AE (%) = [Cu/Si(food) − Cu/Si(feces)/Cu/Si(food)] × 100.

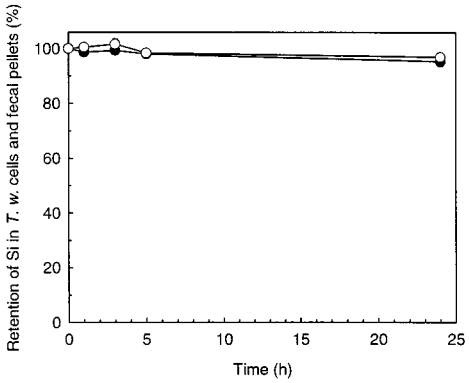

FIGURE 3. Retention of silicon in *T. weissflogii* cells (●) and copepod fecal pellets (○) resuspended in feeding medium (SOW). Mean ± SD (n = 3).

"soluble" fraction of cells grown in Provasoli media and 66% of intracellular Cu in the soluble pool of "Cu-supplemented cells". Since the growth rates and Cu speciation are not reported in this paper, it is difficult to directly compare these results with ours.

Assimilation Efficiency of Copper in Copepods. The concentrations of Cu and Si in the diatoms fed to the copepods and in the copepod fecal pellets egested during feeding periods are shown in Table 3. The Cu and Si levels in the fecal pellets are presented as moles of each element collected in each feeding experiment. Since cells were rinsed with EDTA prior to resuspension and the feeding experiments were initiated after 1 h preincubation of *T. weissflogii* in the feeding medium, short-term loss of surface-bound Cu was minimized. About 5% of cellular copper was lost during the 24 h feeding and was corrected for in the estimation of copper assimilation in copepods. Copepod fecal pellets lose ~20% of their initial Cu in 24 h. The Cu contents of fecal pellets produced during 24 h feeding experiments were not corrected for Cu loss because the differences in calculated AEs that result from the maximum such correction are less than those associated with interexperimental variability.

The loss of silicon from *T. weissflogii* cells and fecal pellets during feeding periods (~24 h) is presented in Figure 3. Silica in fecal pellets was relatively stable in the SOW incubation medium, showing ~5% loss in 24 h. The silicon content of *T. weissflogii* was 0.61 ± 0.06 pmol cell^{-1} and did not vary over the 24 h feeding period. Similar silicon stability in diatoms was observed by Tande and Slagstad (*31*), who reported that there was no noticeable change in the cellular Si concentration of *Thalassiosira anguste-lineata* during a 32 h dark incubation in filtered seawater.

The average assimilation efficiency (AE) of copper in copepods fed diatoms grown at pCu = 12.79 (40.3 ± 6.3%; Table 3) was similar to the percentage of Cu in the cytoplasmic fraction of prey diatom cells (~38%; Figure 2). Reinfelder and Fisher (*18, 19*) suggested that marine copepods absorb metals in the cytoplasm of prey cells through a "liquid" digestion strategy, and the direct relationship between cytoplasmic fractionation and assimilation efficiency has been verified for many trace metals. Our results support these earlier studies and suggest that cytological fractionation in phytoplankton cells is important in determining the trophic transfer of Cu as well. The constant proportion of cytoplasmic Cu in *T. weissflogii* grown with a range of free Cu concentrations and [Cu^{2+}]:[Zn^{2+}] ratios indicates that the absolute amount of "assimilable" Cu in *T. weissflogii* increases at least as fast as the cytoplasmic pool of Cu. At free Cu concentrations greater than $10^{-12.8}$ M, the assimilable Cu pool may include noncytoplasmic Cu as well since the average Cu AE in copepods fed diatoms grown at pCu = 10.79 was 50.3 ± 9.7 (significantly different than the Cu AE at pCu = 12.79, p < 0.14; Table 3), while the cytoplasmic fraction remained at 38% (Figure 2).

We are unaware of other measurements of copper AEs in any aquatic herbivore. We can, however, compare copper assimilation in copepods with that of other metals. The assimilation efficiency of ingested copper (40−50%) was less than that of Se and Zn (50−60%) and higher than that of Cd (37%) or Co and Ag (14−19%) for copepods fed on the same species of diatom (*37*). Copper was also assimilated by copepods fed *T. weissflogii* with a higher efficiency than was Fe (~10% (*20*)) in copepods fed the diatom *T. pseudonana*. Generally, essential trace elements such as Zn and Se were shown to be more enriched in the cytoplasm of algal cells and thus have greater assimilation in copepods than nonessential trace elements (Ag, Am) which were mostly bound to the cell membrane or surface (*18*). Unlike other essential trace elements, Fe is concentrated mainly in the membrane fraction of diatoms (*20*). Based on our study of a single species, the cytoplasmic fractionation of copper appears to be similar to Cd and intermediate with respect to essential and nonessential trace elements.

Reinfelder and Fisher (*18, 19*) suggested that the cytoplasmic distribution of some elements, and thus their AEs in copepods, varied with the physiological state of the algal cells. For instance, they showed that the cytoplasmic fractionation and AE of Zn was higher in stationary-phase than in log-phase cells. The cytoplasmic fraction of copper in *T. weissflogii* was greater (p < 0.056) in stationary phase cells (47.3 ± 5.8) than in exponential phase cells (38.2 ± 5.0%; Table 1). Thus, growth phase may have a modest (9%)

effect on the intracellular partitioning of Cu in marine diatoms and its assimilation in copepods.

Our results suggest that the AE of Cu in herbivorous zooplankton is constant over most of the range of Cu^{2+} concentrations found in natural waters but may be somewhat higher in animals that ingest cells growing at higher Cu concentrations ($pCu \leq 10.8$). Moreover, our findings show that the absolute amount of trophically available Cu in diatoms increases nonlinearly with free Cu concentrations which in coastal waters can increase dramatically in response to a modest increase in total Cu concentration due to the titration of natural organic ligands (*2*, *14*). An increase in free Cu concentration may therefore result in greater biological impacts on phytoplankton and higher trophic transfer of copper in coastal marine food webs than would be predicted based on linear bioaccumulation models.

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