

## Copper uptake kinetics in diverse marine phytoplankton

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### Abstract

We measured short-term uptake rates of copper using the gamma-emitting radioisotope <sup>67</sup>Cu in seven algal species in natural and artificial seawater. Cellular net uptake of Cu was typically rapid over periods of 2–20 min. Net uptake ceased after about 60 min. The most copper-sensitive species examined, *Synechococcus* sp., exhibited 2–3 orders of magnitude higher carbon and surface-area-normalized Cu-accumulation rates (46  $\mu\text{mol Cu mol C}^{-1} \text{min}^{-1}$  and 1,100  $\text{zmol Cu } \mu\text{m}^{-2} \text{min}^{-1}$ ) (zmol =  $10^{-21}$  moles) than those measured in diatoms, chlorophytes, a dinoflagellate, and a coccolithophore. Cu-accumulation rates for *Thalassiosira weissflogii* were three times faster in natural seawater than in EDTA-buffered artificial seawater containing an inorganic Cu concentration of 28  $\text{pmol L}^{-1}$ . Calculations showing that the diffusive flux of inorganic Cu was insufficient to account for observed short-term uptake rates suggest that some of the Cu bound to naturally occurring organic ligands is released through the rapid dissociation of those complexes in the cell's boundary layer.

As with other metals, Cu is essential for the function of a number of enzymes involved in oxygen chemistry and redox reactions (e.g., cytochrome oxidases and superoxide dismutase), but becomes toxic when cellular concentrations are elevated (Williams and Da Silva 1996; Raven et al. 1999). Its unique chemical properties—the ability to form a reduced ion ( $\text{Cu}^+$ ) that forms stable complexes facilitating electron transfer—make Cu well suited for some biological functions. Over the past half billion years, dissolved Cu concentrations have increased in the ocean as the build-up of  $\text{O}_2$  has accelerated the release of Cu from otherwise insoluble  $\text{CuS}$  (Williams and Da Silva 1996). On shorter time scales (hundreds of years), there has been an increase in dissolved Cu concentrations found in coastal systems as a result of anthropogenic inputs, particularly from industry. As a result of changing ocean chemistry on a range of time scales, marine microalgae have had to find ways to cope with excess dissolved Cu in seawater (total dissolved Cu concentrations of  $10^{-9}$  to  $10^{-8} \text{ mol L}^{-1}$ ) relative to their biological requirements ( $10^{-21}$  to  $10^{-18} \text{ mol Cu cell}^{-1}$ ) and maintain intracellular pools of Cu at nontoxic levels. Their ability to do so appears to be taxa specific (Brand et al. 1986).

Strategies evolved by phytoplankton to preserve low intracellular concentrations of metals include (i) biomethylation and transport through cell membranes of metal alkyl compounds by diffusion controlled processes, (ii) the biosynthesis of intracellular polymers that serve as traps for the removal of metal ions from solution, (iii) the sequestration of metal ions to cell surfaces, (iv) the precipitation of insoluble metal complexes (e.g., metal sulfides), and (v) metal exclusion from cells (Davis 1976; Foster 1977; Williams and Da Silva 1996; Whitfield 2001). While biomethylation appears to be limited to nonessential metals (e.g.,  $\text{Hg}^{\text{II}}$ ), the other strategies are pertinent to our understanding of Cu accumulation and homeostasis in algal cells. A considerable literature exists on the toxicity and bioaccumulation of Cu in phytoplankton, but only a few studies have addressed the kinetics of Cu uptake and release in these cells (Hill et al. 1996; Knauer et al. 1997; Croot et al. 2003). Tracer studies are further limited because a long-lived Cu radioisotope or a stable isotope of low mass abundance is not readily available.

Studies of Cu accumulation have revealed that Cu ions bind specialized transport ligands associated with the cell membrane. Cu uptake is light and ATP dependent (Verma and Singh 1990) as well as temperature dependent (Hill et al. 1996), and follows Michaelis–Menten kinetics for facilitated and active transport (Sunda and Huntsman 1995; Hill et al. 1996). The Cu transport rate is thought to be determined by the activity of the free metal ion in the medium and not the total complexed Cu concentration (Sunda 1994; Van Leeuwen 1999). Knauer et al. (1997) found that Cu uptake is mediated by two systems in the freshwater chlorophyte *Scenedesmus subspicatus*: a high-affinity and a low-affinity system operating at  $\text{pCu } 14\text{--}12$  and  $\text{pCu } <12$ , respectively.

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Understanding the interaction of metals with cell surfaces can reveal the mechanisms of metal internalization, nutrition, and toxicity. Papers by others (Van Leeuwen 1999; Galceran et al. 2004; Slaveykova and Wilkinson 2005) have endeavored to refine our understanding of complex reactions governing trace-metal interactions with aquatic biota and the mechanistic interactions at biological surfaces. Here, we report the results of direct measurements of short-term copper uptake in which we measured the Cu accumulation rates with the radioisotope  $^{67}\text{Cu}$  in seven species of marine microalgae from five taxonomic groups. We hypothesize that differences in the cell surface reactivity for Cu may contribute to differences in algal sensitivity to this metal.

*Synechococcus* sp., *Pryamimonas parkeae*, *Tetraselmis levis*, *Amphidinium carterae*, *Emiliania huxleyi*, *Thalassiosira pseudonana*, and *Thalassiosira weissflogii* (Table 1) were grown in Aquil, which consists of synthetic ocean seawater (SOW) and major nutrients (N, P, and Si) chelexed to remove trace-metal impurities (Price et al. 1988/89). This was amended with filter-sterilized (0.2  $\mu\text{m}$ , acid-leached filters) vitamins and trace metals prepared according to the 100  $\mu\text{mol L}^{-1}$  EDTA recipe of Price et al. (1988/89), with only two exceptions: 15  $\text{nmol L}^{-1}$  Cd was added and the final Fe concentration was lowered to 84  $\text{nmol L}^{-1}$  (Quigg et al. 2003). Polycarbonate bottles for media preparation, culture growth, and sample manipulations were prepared using trace-metal clean procedures (Bruland et al. 1979). This involved leaching all materials in 1  $\text{mol L}^{-1}$  HCl (reagent grade in Milli-Q water; 18.2 mega $\Omega$ ) at 60°C for at least 24 h and then rinsing five times with Q-water prior to use.

Algal cultures (Table 1) were grown axenically at 19°C  $\pm$  1°C using a 12:12 light:dark cycle. Examination of bacteria in the cultures with DAPI staining revealed an insignificant quantity of bacteria in the cultures (<3% of the total biomass). Cool-white fluorescent bulbs supplied 250  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (measured in situ with a 4-pie collector light meter; QSL-100 Biospherical Instruments). Cultures were maintained in exponential growth through at least 5 generations with a pCu of 13.79 before starting  $^{67}\text{Cu}$ -accumulation experiments. Growth rates were assessed from daily counts made with a calibrated Coulter Multisizer particle counter, which we also used to measure cell volumes for all species except *Synechococcus* sp. (Table 1). The quantum yield of photochemistry (Fv/Fm), where Fv = variable fluorescence and Fm = maximal fluorescence, was measured with a Fast repetition rate fluorometer (Kolber et al. 1998). All cultures were healthy (Fv/Fm = 0.55–0.70) under the experimental conditions. Cells were harvested from exponentially growing, optically thin cultures for Cu-accumulation experiments.

The gamma-emitting radionuclide  $^{67}\text{Cu}$  ( $t_{1/2} = 2.58$  d; stock specific activity 87.7 MBq  $\mu\text{g}^{-1}$ ) was prepared at the Brookhaven National Laboratory (New York). Seawater (S = 30) was collected in an acid-washed carboy from Sandy Hook (New Jersey) several days before starting experiments, passed through 0.2- $\mu\text{m}$  polycarbonate filters, and stored in the dark at 4°C. In order to ensure that the specific activity of  $^{67}\text{Cu}$  in the uptake media was constant in all Cu pools, particularly organic complexes that exchange slowly with inorganic Cu, we equilibrated 225-mL aliquots of Sandy

Table 1. Cellular characteristics of the microalgae investigated: phylogenetic affiliation, clone, cell volume and surface area, and C and Cu quotas. The average cell density of each culture during the accumulation experiments is included for reference.

	Clone	Cell volume ( $\mu\text{m}^3$ , $\pm$ SD)	Cell surface area ( $\mu\text{m}^2$ )	Cell radius* ( $\mu\text{m}$ )	Cell C quota ( $\text{pmol cell}^{-1}$ )	Cell Cu quota ( $\text{amol cell}^{-1}$ )	Cell concentration ( $\text{cells L}^{-1}$ )
Cyanophyceae							
<i>Synechococcus</i> sp.	CCMP 1334	1.2 (0.4)	4.7	0.77	0.13 $\ddagger$	0.2 $\ddagger$	$2.4 \times 10^8$
Chlorophyceae							
<i>Pryamimonas parkeae</i>	CCMP 724	590 (35)	340	5.2	3.9 $\ddagger$	11 $\ddagger$	$2.5 \times 10^6$
<i>Tetraselmis levis</i>	CCMP 896	270 (12)	200	4.1	7.6 $\ddagger$	18 $\ddagger$	$3.9 \times 10^6$
Dinophyceae							
<i>Amphidinium carterae</i>	CCMP 1314	510 (25)	310	4.9	6.6 $\ddagger$	2.9 $\ddagger$	$3.2 \times 10^6$
Prymnesiophyceae							
<i>Emiliania huxleyi</i>	ASM1	140 (7)	130	3.2	1.4 $\ddagger$	0.92 $\ddagger$	$1.4 \times 10^6$
Bacillariophyceae							
<i>Thalassiosira pseudonana</i>	CCMP 1335	78 (5)	88	2.7	0.45 $\S$	3.8 $\S$	$2.8 \times 10^6$
<i>Thalassiosira weissflogii</i>	CCMP 1336	930 (12)	460	6.1	11 $\ddagger$	21 $\ddagger$	$2.3 \times 10^6$

CCMP, Culture Collection of Marine Microalgae (Bigelow Laboratory for Ocean Sciences, USA); ASM1, provided courtesy of Dr. Ian Probert (Algalbank, Université de Caen Basse Normandie, France).

\* These are estimates of cell radius based on measurements of cell size made with a calibrated Multisizer Coulter counter.

$\ddagger$  Estimated by assuming the relationship between cell size and C or Cu quota has an exponent of 2.25 (Hudson and Morel 1993).

$\S$  Quigg et al. (2003).

$\S$  Sunda and Huntsman (1995).

Hook seawater (SHSW) with  $^{67}\text{Cu}$  (237 kBq) for 24 h prior to starting experiments.

Initial experiments were performed with *T. weissflogii* in order to define a suitable time period for measuring  $^{67}\text{Cu}$ -accumulation rates and to examine some of the important influences on Cu-accumulation kinetics. For these experiments, Cu uptake was measured in *T. weissflogii* exposed to stable inorganic Cu in SOW (no EDTA), to  $^{67}\text{Cu}$ -equilibrated Aquil (pCu = 13.79) and  $^{67}\text{Cu}$ -equilibrated SHSW. For the inorganic Cu-uptake experiment, *T. weissflogii* was grown in natural seawater (Tuckerton, New Jersey) supplemented with 1/10 Aquil concentration of Fe and Zn, but no added nutrients, vitamins, EDTA, or other metals. The total dissolved Cu concentration in the Tuckerton seawater (22.5 nmol L<sup>-1</sup>) was determined by graphite furnace atomic absorption spectrometry (GF-AAS) after preconcentration (Bruland and Coale 1985). Cells were then transferred to SOW (no EDTA) and 20 nmol L<sup>-1</sup> Cu added. Cu uptake was followed over 4 h.  $^{67}\text{Cu}$  accumulation was also measured over 20 min in *T. weissflogii* grown in Aquil and then re-suspended in both  $^{67}\text{Cu}$ -equilibrated Aquil (5  $\mu\text{mol L}^{-1}$  EDTA) with a pCu of 13.79 and  $^{67}\text{Cu}$ -equilibrated SHSW. These two latter treatments will be referred to as *T. weissflogii*-Aquil and *T. weissflogii*, respectively, and will be used to assess differences, if any, in the availability of Cu ions in the presence of an artificial chelator versus natural chelators. Based on preliminary experiments and the work of Knauer et al. (1997), a 20-min time period was considered suitable for measuring Cu-accumulation rates.

In short-term  $^{67}\text{Cu}$ -accumulation experiments, algal cells were concentrated by centrifugation at 10,000  $\times$  g (14°C) 12 h prior to the commencement of the  $^{67}\text{Cu}$ -accumulation experiments and re-suspended in 75 mL of unlabeled SHSW.  $^{67}\text{Cu}$ -accumulation assays were performed at 20°C and initiated by the addition of 25-mL aliquots of exponentially growing cells into 225 mL of  $^{67}\text{Cu}$ -SHSW. At 1-, 2-, 5-, 10-, 15-, and 20-min intervals, 40-mL suspensions were filtered under gentle vacuum (<20 kPa) onto 0.4–3- $\mu\text{m}$  (dependent on cell size) polycarbonate membranes and rinsed with 5 mL SHSW. The vacuum was turned off and the filtered cells were covered with 10 mL of 10 mmol L<sup>-1</sup> EDTA in SOW (ion-exchange resin treated, pH 8) for 2.5 min. The vacuum was turned on again, and a second 5-mL SHSW wash completed the rinsing procedure. This treatment has previously been shown to effectively remove loosely bound Cu from diatoms (Chang and Reinfelder 2000). Filters were transferred directly to tubes for counting in a Pharmacia-Wallac LKB gamma counter with a well-type NaI (TI) detector at 185 keV; samples were counted for 5 min, with propagated counting errors <5%.

At the end of each  $^{67}\text{Cu}$ -accumulation experiment, triplicate 1-mL aliquots were taken to measure the total radioactivity in suspension for each culture. Aliquots (1 mL) were also taken for cell counts. Lugols' iodine solution was used to kill the cells, samples were then stored in the dark at 4°C until they could be counted with a hemocytometer. The total dissolved Cu concentration in the SHSW was 6.8 nmol L<sup>-1</sup> (determined by GF-AAS). The specific activity of  $^{67}\text{Cu}$  in the experimental seawater was calculated as the total cpm L<sup>-1</sup> of  $^{67}\text{Cu}$  in each bottle divided by the total dissolved Cu

concentration in SHSW plus 0.24 nmol L<sup>-1</sup> from the radio-tracer addition (total of 7.0 nmol L<sup>-1</sup>). The Cu contents of cells were quantified as the background and filter blank-corrected cpm measurements of each filter divided by the specific activity of  $^{67}\text{Cu}$  and the number of cells collected.  $^{67}\text{Cu}$ -accumulation experiments were conducted in duplicate for all species except *T. pseudonana*, for which we did triplicate measurements.

$^{67}\text{Cu}$ -accumulation rates were determined by linear regressions of the initial linear portions of the  $^{67}\text{Cu}$ -uptake curves. In cases where  $^{67}\text{Cu}$  accumulation was delayed or appeared to reach steady state sooner than 20 min, we calculated accumulation rates over a shorter period: 2–15 min for *P. parkeae* and *T. weissflogii* and 2–20 min for *Synechococcus* sp., *E. huxleyi*, and *T. weissflogii*-Aquil. The 10-min time points of both replicates for *A. carterae* showed very high  $^{67}\text{Cu}$  accumulation; their exclusion did not significantly affect the calculated slope ( $p < 0.05$ ).

The maximum diffusive flux of Cu from the bulk solution to the surface of a spherical cell ( $J_D$ ) is a function of the cell radius,  $r$  (cm), the diffusion coefficient of dissolved Cu ( $D$  at 20°C,  $3.6 \times 10^{-4}$  cm<sup>2</sup> min<sup>-1</sup>; Hudson and Morel 1993) and the concentration of dissolved inorganic Cu species ( $\text{Cu}'$ ; mol L<sup>-1</sup>); that is,  $J_D = 4\pi r D \text{Cu}'$  (mol cell<sup>-1</sup> min<sup>-1</sup>). We used the total inorganic Cu concentration to represent the labile Cu pool although weak organic Cu complexes may also be labile.  $\text{Cu}'$  was estimated to be 28 pmol L<sup>-1</sup>, assuming that 99.6% of the total dissolved Cu in Sandy Hook seawater was organically complexed (Sunda et al. 1987; Donat and Bruland 1995). This is consistent with our recent measurements of  $\text{Cu}^{2+}$  in the New York Bight by flow-through Cu ISE, which average  $10^{-11.5}$  mol L<sup>-1</sup> ( $\text{Cu}'$  of 30 pmol L<sup>-1</sup>).

Cu uptake over 5 h for *T. weissflogii* was greatest in the initial 30 min (1.2 amol Cu cell<sup>-1</sup> min<sup>-1</sup>, or 0.12  $\mu\text{mol Cu mol C}^{-1}$  min<sup>-1</sup>, or 2.6 zmol Cu  $\mu\text{m}^{-2}$  min<sup>-1</sup>), then slowed until an apparent equilibrium was reached at 60 min with respect to Cu partitioning between dissolved and particulate phases (Fig. 1). Based on these findings and those from previous studies, further  $^{67}\text{Cu}$ -accumulation experiments were limited to 20 min.

Results of the short-term  $^{67}\text{Cu}$ -accumulation experiments performed in SHSW are presented in Fig. 2 for all species except *T. weissflogii*. Accumulation of Cu approached equilibrium in just 20 min for *Synechococcus* sp., *A. carterae*, *P. parkeae*, and *E. huxleyi*, but not for *T. levis* and *T. pseudonana* (Fig. 2). Using the linear portions of these  $^{67}\text{Cu}$ -accumulation curves, we calculated Cu-uptake rates of 0.03–46  $\mu\text{mol Cu mol C}^{-1}$  min<sup>-1</sup>, or 0.7–1,100 zmol Cu  $\mu\text{m}^{-2}$  min<sup>-1</sup> (Table 2). Cu-accumulation rates measured in the chlorophytes, diatoms, a dinoflagellate, and a coccolithophore are several orders of magnitude slower than those measured for the cyanobacterium (Table 2; Fig. 2). Interspecies differences in Cu-uptake rates were not linearly related to differences in cell surface area (Table 2) or the geographic location each species would have typically occupied in the marine environment. For example, Cu-accumulation rates for the coastal *P. parkeae* and estuarine *T. levis* are similar.

Experiments with *T. weissflogii* comparing Cu uptake in Aquil and in natural seawater showed that rapid uptake oc-

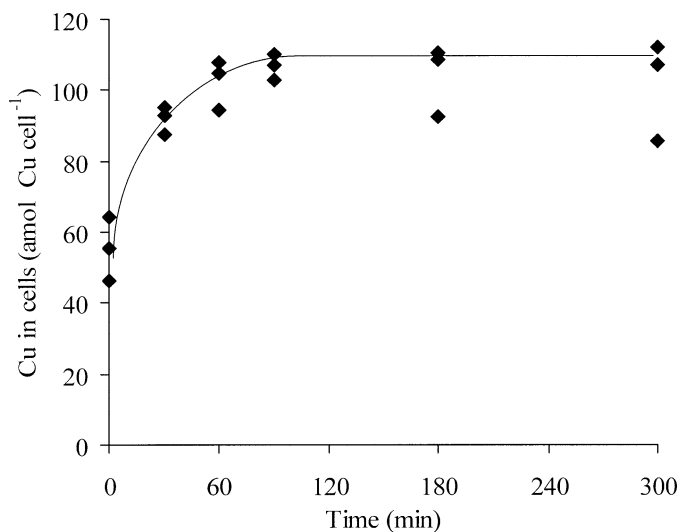


Fig. 1. *T. weissflogii* was grown in coastal seawater from 5 miles off Tuckerton, New Jersey, which had  $22.5 \text{ nmol L}^{-1}$  total dissolved Cu. To this, we added known concentrations of Fe and Zn (see Methods). In order to measure Cu-accumulation rates, cells were transferred to synthetic ocean water without EDTA and  $20 \text{ nmol L}^{-1}$  inorganic Cu (see Methods). Results are shown for replicate determinations at each time point.

occurred in both cultures over the first minute (Fig. 3), followed by slower uptake over the next 15 min. This latter uptake rate was significantly faster in *T. weissflogii* cells in natural seawater than those in Aquil. Indeed, short-term uptake rates for all species in natural seawater exceeded the calculated maximum diffusion fluxes of inorganic Cu ( $\text{Cu}'$ ) by factors of four to more than 600 (Table 2). This result indicates the presence of a kinetically labile and bioavailable pool of Cu organic complexes in these coastal surface waters that dissociates on the time scale of minutes. This kinetically labile Cu is approximately 10 times larger than the inorganic pool for eukaryotic microalgae and more than 100 times larger for cyanobacteria.

The patterns of Cu uptake displayed by most of the algal species during the 20-min exposures revealed a rapid uptake within the first minute, followed by a slower uptake over a 15–20-min period. Similar patterns and rates of Cu uptake were observed in the freshwater chlorophytes *Chlamydomonas reinhardtii* (Hill et al. 1996) and *Scenedesmus subspicatus* (Knauer et al. 1997) and the marine cyanobacterium *Synechococcus* sp. (Croot et al. 2003), all of which were washed with EDTA. Uptake of Cu during the first minute is most likely due to adsorption of Cu to cell surfaces, possibly depleting each cell's boundary layer of  $\text{Cu}'$ . Indeed, approximately 40% of the initial  $\text{Cu}'$  ( $12 \text{ pmol L}^{-1}$ ) was removed by the diatoms *T. pseudonana* and *T. weissflogii* in the first minute. Thereafter, the dissociation of organic Cu complexes, adsorption, internalization, and Cu efflux would all contribute to the net uptake rate observed for all species. It is noteworthy that the association of Cu with the cells, including that bound to cell-surface sites, was strong enough to withstand EDTA washes.

Cu accumulation in algal cells can be modeled as the binding of Cu to a cell surface transport ligand followed by

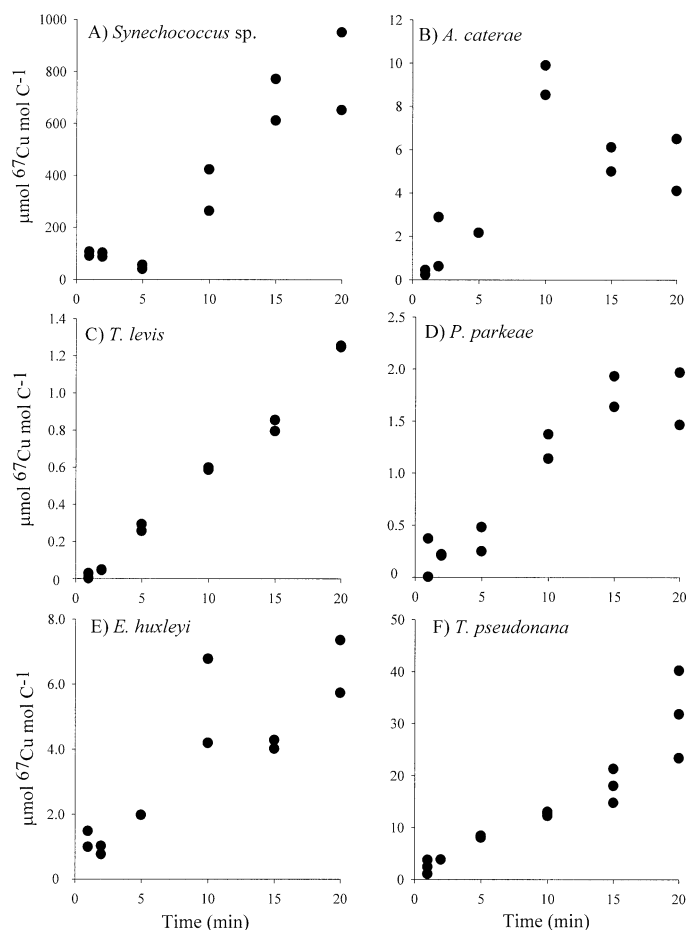


Fig. 2. Accumulation of Cu ( $\mu\text{mol Cu mol C}^{-1}$ ) as a function of time (minutes) measured for (A) *Synechococcus* sp., (B) *A. catenae*, (C) *T. levis*, (D) *P. parkeae*, (E) *E. huxleyi*, and (F) *T. pseudonana* in SHSW labeled with  $^{67}\text{Cu}$ . Results are shown for replicate determinations at each time point.

transport across the cell membrane (Hudson and Morel 1993). Most cell-surface accumulation, however, is the result of nonspecific binding to a variety of chemical functional groups on cell surfaces (Crist et al. 1994) and does not lead directly to transport. Thus, the rapid adsorption of Cu to algal cell surfaces observed in *S. subspicatus* (Knauer et al. 1997), *Dunaliella tertiolecta* (Gonzalez-Davila et al. 2000), and *E. huxleyi* (Vasconcelos and Leal 2001) that occurs within seconds of a Cu perturbation does not represent internalization. The short lag in Cu accumulation following the initial rapid uptake within the first minute may represent the diffusion and dissociation of organic Cu complexes in the cell's boundary layer or the turnover of metal transport ligands on the cell's surface.

For *T. weissflogii*,  $^{67}\text{Cu}$ -accumulation rates were much faster for cells in SHSW than in Aquil, indicating that the free Cu concentration in SHSW was  $>10^{-13.9} \text{ mol L}^{-1}$ , consistent with our recent observations, or that natural organic ligands present in SHSW were more kinetically labile than artificial complexes or more reactive with cell surface functional groups of *T. weissflogii*. Moreover, calculated diffusive fluxes of inorganic Cu were lower than the observed

Table 2. Measured short-term Cu accumulation rates ( $\rho$ ) for the seven algal species expressed per cell, per unit biomass (carbon), and per cell surface area. The maximum diffusive flux of inorganic Cu ( $\text{Cu}'$ ) was calculated as described in the text. For the diffusion calculations, we estimated  $\text{Cu}'$  (28  $\text{pmol L}^{-1}$ ) using the measured total Cu concentration in SHSW (7.0  $\text{nmol L}^{-1}$ ) and assuming 0.4% of the total Cu is present as  $\text{Cu}'$  in coastal seawater (Donat and Bruland 1995). The maximum diffusive flux was estimated from the ratio ( $\rho: J_D$ ) of measured Cu accumulation rates to the calculated maximum diffusion rates per cell.

	Cu accumulation rate ( $\rho$ )*			Max $\text{Cu}'$ diffusion rate ( $J_D$ ) ( $\text{zmol cell}^{-1} \text{min}^{-1}$ )	Ratio $\rho: J_D$
	$\text{amol Cu cell}^{-1} \text{min}^{-1}$	$\mu\text{mol Cu mol C}^{-1} \text{min}^{-1}$	$\text{zmol Cu } \mu\text{m}^2 \text{min}^{-1}$		
<b>Cyanophyceae</b>					
<i>Synechococcus</i> sp.	6.1	46	1100	10	610
<b>Chlorophyceae</b>					
<i>Pyramimonas parkeae</i>	0.50	0.13	1.5	66	8
<i>Tetraselmis levis</i>	0.48	0.064	2.4	51	9
<b>Dinophyceae</b>					
<i>Amphidinium carterae</i>	2.0	0.29	6.3	63	31
<b>Prymnesiophyceae</b>					
<i>Emiliania huxleyi</i>	0.42	0.30	3.2	41	10
<b>Bacillariophyceae</b>					
<i>Thalassiosira pseudonana</i>	0.80	1.8	9.1	34	24
<i>Thalassiosira weissflogii</i>	0.33	0.03	0.72	77	4
<i>Thalassiosira weissflogii</i> †	0.094	0.01	0.21	0.6	188

\* Accumulations rates were calculated over the following time periods: 0–20 min for *T. levis* and *A. carterae*, 2–15 min for *P. parkeae* and *T. weissflogii*, 2–20 min for *Synechococcus* sp., *E. huxleyi*, and *T. weissflogii*-Aquil.

short-term Cu-accumulation rates (Table 2). Cu accumulation does not strictly follow the free-ion activity model. For example, Cu uptake appears to be diffusion limited for freshwater periphyton communities (Meylan et al. 2003; Meylan et al. 2004).

Vasconcelos and Leal (2001) found that uptake rates in *E. huxleyi* were dependent on both the speciation and source of organic ligands (natural vs. synthetic) of Cu. While many studies have assessed the influence of artificial chelators on

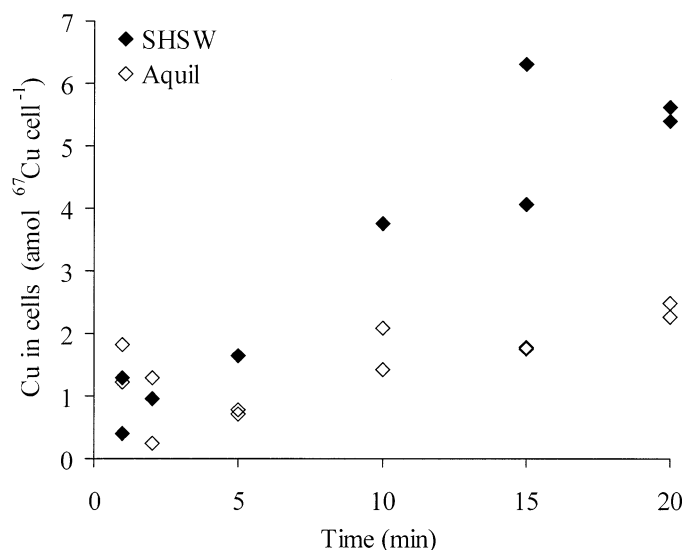


Fig. 3. Cu-accumulation rates ( $\text{amol } ^{67}\text{Cu cell}^{-1}$ ) measured for *T. weissflogii* exposed to  $^{67}\text{Cu}$  in natural seawater (SHSW) and artificial seawater-Aquil. Results are shown for replicate determinations at each time point.

Cu bioavailability to algae (usually in culture), relatively few studies have evaluated the influence of naturally occurring dissolved organic matter on the speciation and bioavailability of Cu for microalgae (Fisher and Frood 1980; Mylon et al. 2003). While these latter studies generally confirm the conclusions of the findings with artificial chelators that organically complexed Cu is less available to cells than free Cu (see also Campbell 1995), there are few experimental comparisons, such as the present, directly comparing the bioavailability of Cu exposed to natural and artificial chelators.

Uptake rate:  $\text{Cu}'$  diffusive flux ratios vary as a function of the  $\text{Cu}'$  in seawater and the phytoplankton species in question. We performed a sensitivity analysis (Fig. 4) for the Cu-uptakes rates we measured in coastal seawater. The range of  $\text{Cu}'$  covered is five times lower  $\text{Cu}'$  than in SHSW (28  $\text{pmol L}^{-1}$ ) and up to 25 times higher (Fig. 4). This includes the range of 90% to 99.92% of the total dissolved Cu in SHSW as being organically complexed. When  $\text{Cu}' > 0.14 \text{ nmol L}^{-1}$  (2% of the total), inorganic Cu can supply all of the Cu taken up in most of the eukaryotes, but not in the prokaryote, *Synechococcus* sp. (Fig. 4).

The short-term Cu-uptake rates that we and others have measured are much faster than those that can be computed by multiplying the steady-state Cu quota of cells by their growth rates over periods of days. In our calculations, this apparent discrepancy was most pronounced for *Synechococcus* sp. (data not shown). The difference between short-term and steady-state Cu-uptake rates is probably attributable to a sharp decrease in the net binding rate of Cu to cell surfaces over time (Fig. 1). Additionally, rapid efflux of internalized Cu has been observed in other algal species as well as *Synechococcus* (Verma and Singh 1991; Knauer et al. 1997;

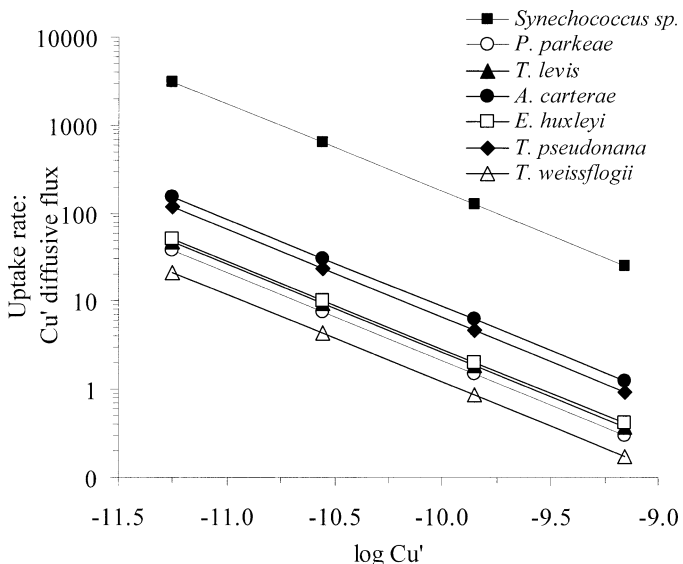


Fig. 4. Sensitivity analysis for uptake rate:Cu' diffusive flux ratios versus the concentration of dissolved inorganic Cu species (Cu') for the rates we measured in *Synechococcus* sp., *A. carterae*, *T. levis*, *P. parkeae*, *E. huxleyi*, *T. pseudonana*, and *T. weissflogii* in coastal seawater. The range of Cu' covers five times lower Cu'ime than in SHSW, 28 pmol L<sup>-1</sup>, and 5 and 25 times higher than 28 pmol L<sup>-1</sup>. This includes the range of 90% to 99.92% of the total dissolved Cu in SHSW as being organically complexed.

Croot et al. 2003). An increase in efflux could contribute to this difference as well.

Among the species examined here, the short-term Cu-uptake rate was greatest for *Synechococcus*. This species has the largest cell surface-to-volume ratio of all the cells considered; Cu binding ( $\mu\text{m}^{-2}$ ) to these cells was also greatest for *Synechococcus*. The high short-term uptake rate of Cu in cyanobacteria may contribute to the marked sensitivity of this group to Cu (Brand et al. 1986). Eukaryotic algae, which evolved and radiated in a Cu-rich ocean (Williams and Da Silva 1996; Falkowski et al. 2004), acquired Cu-tolerance mechanisms that may include a less reactive cell surface, as shown in Table 2. The maximum Cu' diffusion rate is linearly related to the Cu-accumulation rate per surface area (log:log; not shown) as would be predicted from Fick's law. A greater cell size for eukaryotes relative to prokaryotes may have provided an additional selective advantage against toxic metal accumulation in these algae relative to their ancestors in an oxidizing ocean.

Our results build on earlier studies by Brand et al. (1986), Sunda and Huntsman (1995), and, more recently, Croot et al. (2003) and provide additional information for explaining the differential response of diverse algal species to Cu perturbations in the marine environment. In order to understand what governs species-specific Cu acquisition and homeostasis, future studies should focus on Cu-tolerance mechanisms as well as the production of external Cu-binding ligands.

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