

COPPER-UPTAKE KINETICS OF COASTAL AND OCEANIC DIATOMS¹

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We investigated copper (Cu) acquisition mechanisms and uptake kinetics of the marine diatoms *Thalassiosira oceanica* Hasle, an oceanic strain, and *Thalassiosira pseudonana* Hasle et Heimdal, a coastal strain, grown under replete and limiting iron (Fe) and Cu availabilities. The Cu-uptake kinetics of these two diatoms followed classical Michaelis–Menten kinetics. Biphasic uptake kinetics as a function of Cu concentration were observed, suggesting the presence of both high- and low-affinity Cu-transport systems. The half-saturation constants (K_m) and the maximum Cu-uptake rates (V_{max}) of the high-affinity Cu-transport systems (~ 7 – 350 nM and 1.5 – 17 $\text{zmol} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1}$, respectively) were significantly lower than those of the low-affinity systems (>800 nM and 30 – 250 $\text{zmol} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1}$, respectively). The two Cu-transport systems were controlled differently by low Fe and/or Cu. The high-affinity Cu-transport system of both diatoms was down-regulated under Fe limitation. Under optimal-Fe and low-Cu growth conditions, the K_m of the high-affinity transport system of *T. oceanica* was lower (7.3 nM) than that of *T. pseudonana* (373 nM), indicating that *T. oceanica* had a better ability to acquire Cu at subsaturating concentrations. When Fe was sufficient, the low-affinity Cu-transport system of *T. oceanica* saturated at $2,000$ nM Cu, while that of *T. pseudonana* did not saturate, indicating different Cu-transport regulation by these two diatoms. Using CuEDTA as a model organic complex, our results also suggest that diatoms might be able to access Cu bound within organic Cu complexes.

Key index words: copper; Cu; diatom; Fe; iron; kinetics; *Thalassiosira*; transport

Abbreviations: Cu', inorganic Cu; DTPA, diethylenetriaminepentaacetate; fL, 10^{-15} L, μm^3 ; K_m , half-saturation constant; V_{max} , maximum Cu-uptake rate; zmol , 10^{-21} mol

Copper (Cu) is an essential micronutrient for phytoplankton and plays an important role as a cofactor of electron-transfer proteins, such as cytochrome oxidase in the respiratory chain reaction and superoxide dismutase in the detoxification of reactive oxygen species (reviewed by Raven et al. 1999, Merchant et al. 2006). Most recently, Cu has been shown to be involved in the high-affinity iron (Fe) transport system of coastal and oceanic diatoms (Maldonado et al. 2006), as previously shown in *Chlamydomonas* and yeast (Askwith et al. 1994, La Fontaine et al. 2002). In addition to these basic Cu demands of marine diatoms, it has been demonstrated that oceanic diatoms have higher Cu requirements than coastal strains, possibly due to the replacement of Fe-containing enzymes by Cu-containing equivalents (Annett et al. 2008). For example, in *T. oceanica*, the Cu-containing enzyme plastocyanin—which is found in all higher plants, green algae, and some blue-green algae—replaces the Fe-containing enzyme, cytochrome c_6 , in photosynthesis (Peers and Price 2006). This replacement lowers Fe demand by 10% and may partly explain the success of oceanic phytoplankton in open-ocean waters, where Fe concentrations are too low to support the growth of coastal isolates (Peers and Price 2006). Conversely, Cu is considered toxic and can damage a cell when the intracellular Cu content is excessive (Rueter et al. 1979, Moffett et al. 1997, Sunda and Huntsman 1998, Raven et al. 1999). Thus, the mechanisms of intracellular Cu homeostasis—controlling Cu uptake, intracellular distribution, utilization, and detoxification—have been the subject of considerable interest in yeast, higher plants, and freshwater microalgae (Clemens 2001,

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Puig and Thiele 2002, Merchant et al. 2006). As of yet, there is little information on the mechanisms of Cu internalization and homeostasis in marine phytoplankton.

As observed for other trace elements, the concentration of Cu in the sea tends to increase from open-ocean to coastal waters (Boyle et al. 1981, Rutgers van der Loeff et al. 1997). Total dissolved Cu concentrations in nonpolluted surface open-ocean waters range from 0.5 to 6 nM, with an average concentration of 2 nM (Bruland and Franks 1983, Coale and Bruland 1988, Jickells and Burton 1988, Moffett 1995, Rutgers van der Loeff et al. 1997, Moffett and Dupont 2007). In comparison, Cu concentrations in coastal waters range from 2 to 150 nM (Sunda et al. 1990, Moffett et al. 1997, Buck et al. 2007), depending on varying degrees of contamination by anthropogenic Cu sources. In natural seawater, dissolved Cu species include free Cu^{2+} ions, inorganic complexes, and organic Cu complexes, though organic complexation dominates. Indeed, up to 99.8% of dissolved Cu is bound to the strong organic ligand class (L1) in the open ocean (Coale and Bruland 1988, 1990) and in coastal waters (Buck et al. 2007). In addition, changes in Cu speciation in coastal waters may be observed seasonally due to variations in phytoplankton community composition (Croot 2003) or regionally due to the absence of strong organic ligands (Moffett et al. 1997). Due to the difference in Cu concentrations in the open ocean and in coastal waters, Cu-uptake mechanisms of phytoplankton inhabiting these contrasting environments may also vary.

Many studies have investigated Cu-uptake kinetics and transport in yeast (reviewed by Puig and Thiele 2002, Kim et al. 2008) and freshwater green algae (reviewed by Merchant et al. 2006). In yeast, both high- and low-affinity Cu-transport systems have been identified (Dancis et al. 1994a, Hassett et al. 2000). The high-affinity system is energy dependent and specific for Cu^+ over other metal ions (Lin and Kosman 1990, Dancis et al. 1994a); its K_m is $\sim 4 \mu\text{M}$ dissolved Cu (DeRome and Gadd 1987, Lin and Kosman 1990, Dancis et al. 1994a). In contrast, *Chlamydomonas* only exhibits a high-affinity Cu-transport system, with an invariant K_m of $0.2 \mu\text{M}$ dissolved Cu and a variable V_{max} depending on Cu availability during growth (Hill et al. 1996). These studies illustrate the variety of microbial Cu-transport pathways and physiological responses to Cu availability.

In this study, we investigated the short-term Cu-uptake kinetics of one oceanic diatom, *T. oceanica*, and one coastal diatom, *T. pseudonana*, grown under various nontoxic Fe and Cu levels. Our goal was to investigate the existence of a low- and a high-affinity Cu-transport system in these organisms and determine what controls the substrate affinity and maximum transport velocity. Historically, the lack of

suitable Cu radiotracers has posed a significant limitation to the study of Cu-transport kinetics. In collaboration with the Canadian National Particle and Nuclear Physics Laboratory at Tri-University Meson Facility campus (TRIUMF) in Vancouver, British Columbia, Canada, we have gained access to a regular supply of the short-lived ^{64}Cu and ^{67}Cu isotopes. The use of these isotopes in short-term uptake experiments allowed us to do extensive research on Cu uptake that would be very difficult to obtain with other methods.

MATERIALS AND METHODS

Study organisms. Two centric diatoms, *T. oceanica* (CCMP 1003) and *T. pseudonana* clone 3H (CCMP1335), isolated from oceanic and coastal environments, respectively, were used in this study. Cultures were obtained from the Bigelow Laboratory for Ocean Sciences (West Boothbay Harbor, ME, USA). The mean cell diameters of *T. oceanica* and *T. pseudonana* grown under nonlimiting trace-metal conditions were ~ 6.5 and $4.6 \mu\text{m}$, respectively.

Media and culture manipulations. Axenic diatoms were grown as semicontinuous batch cultures in the chemically well-defined artificial seawater medium AQUIL (Price et al. 1989) at $19 \pm 1^\circ\text{C}$. To eliminate circadian rhythms of phytoplankton when grown under a day-night light cycle, our cultures were grown under continuous illumination with cool-white fluorescent lights ($150 \mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; Sylvania, Mississauga, Canada), allowing us to monitor and execute experiments at any time of day.

Except for the addition of Fe and Cu, the AQUIL medium used in this study was identical in chemical composition to that described by Maldonado et al. (2006). Four culture media treatments were prepared for each species with various additions of Fe and/or Cu (Table 1). Sterile, trace-metal-clean techniques were used during all experiments and manipulations.

Determination of growth rates and cell sizes. Cells were acclimated to various Fe and/or Cu culture conditions in 28 mL polycarbonate tubes. In vivo fluorescence was measured daily with a Turner 10-AU Fluorometer (Turner Designs, Sunnyvale, CA, USA). The ln of fluorescence versus time was used to calculate growth rates (d^{-1}). Cultures were considered acclimated when growth rates of five successive transfers varied by $<15\%$ (Brand et al. 1981). For the Cu-uptake kinetic experiments, acclimated cultures in midexponential phase were transferred from 28 mL tubes to 2 L bottles. The growth rates of the diatoms in these bottles were monitored daily. Cell density and size (μm) were also determined for live samples using a Coulter Z2 Particle Count and Size Analyzer (Beckman Coulter Inc., Brea, CA, USA). Cell surface area (μm^2) and volume ($\text{fL} = 10^{-15} \text{L}$) were calculated assuming a spherical cell shape.

Measurement of Cu-uptake rates. For all experiments, 2 L cultures of various Cu/Fe treatments were grown as indicated above. Nonradioactive Cu and a spike of the carrier-free tracer ^{67}Cu ($0.32 \text{ MBq} \cdot \text{L}^{-1}$) were buffered with $100 \mu\text{M}$ EDTA in 125 mL chelexed synthetic ocean water (SOW) containing $100 \mu\text{M}$ Si, $10 \mu\text{M}$ P, and no NO_3^- , trace metals, or vitamins. Given that EDTA is often used as a ligand in phytoplankton culture media, EDTA was chosen for these experiments. The gamma-emitting radionuclide ^{67}Cu ($t_{1/2} = 62 \text{ h}$) was produced at TRIUMF. Different Cu concentrations were added depending on the experiments (see below). After the Cu and EDTA additions, the media had a pH of 8.1 and was allowed to equilibrate chemically for at least 12 h before use. The next day, trace-metal-clean techniques were used to filter 250 mL

TABLE 1. Culture media with various Fe and Cu additions. The units of total Cu concentration were nM. Metals were added bound to 100 μM EDTA. MINEQL + 4.61 (Environmental Research Software) was used to calculate free metal concentrations ($\text{pM} = -\log [M^+]$, where units of $[M^+]$ are $\text{mol} \cdot \text{L}^{-1}$). In these calculations, we included the Cu contamination of our media (1 nM Cu, Maldonado et al. 2006).

	<i>Thalassiosira oceanica</i>		<i>Thalassiosira pseudonana</i>	
	[Fe] _{total} (pFe)	[Cu] _{total} (pCu)	[Fe] _{total} (pFe)	[Cu] _{total} (pCu)
Fe replete/Cu replete	1,370 (19)	11.2 (14.1)	1,370 (19)	11.2 (14.1)
Fe replete/low Cu	1,370 (19)	1.0 (15.1)	1,370 (19)	1.0 (15.1)
Fe limited/Cu replete	1.28 (22)	11.2 (14.1)	12.5 (21)	11.2 (14.1)
Fe limited/low Cu	1.28 (22)	2.96 (14.8)	12.5 (21)	1 (15.1)

aliquots of mid-log-phase 2 L culture onto trace-metal-clean 2 μm polycarbonate (Poretics, 24 h acid-soaked) filters (47 mm) by gentle vacuum (<100 mm Hg) and washed with 10 mL chelexed SOW. Each filter was immediately resuspended in 125 mL of seawater containing the desired experimental ratio of ^{67}Cu to nonradioactive Cu concentration/treatment. Although short-term Cu uptake by marine phytoplankton is not a light-dependent process (Gnassia-Barelli and Hardstedt-Romeo 1982), the cells were incubated at room temperature and exposed to 150 $\mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ after the resuspension. The initial sampling time was 10 min after resuspension, followed by 15–30 min sampling intervals. Twenty-five milliliter aliquots of culture were removed during each sampling interval. Cells were vacuum filtered onto a 2 μm 25 mm polycarbonate membrane. The filtered cells were soaked for 5 min with 5 mL of 1 mM diethylenetriaminepentaacetic acid (DTPA) solution (dissolved in sterile SOW, pH adjusted to 8.14; Croot et al. 1999) to bind the extracellular adsorbed ^{67}Cu , and then washed with 5 mL SOW. The radioactive filters were placed in scintillation vials, and radioactivity was determined using a PerkinElmer 1480 WIZARD 3rd Gamma Counter (PerkinElmer Inc., Waltham, MA, USA). Duplicate initials (1 mL) of the uptake medium were also taken to determine the specific activity of ^{67}Cu in the uptake media (MBq $^{67}\text{Cu}/\text{Cu}$ concentration added). At the end of each ^{67}Cu -uptake experiment, a sample was fixed with Lugol's solution to determine cell density using the Coulter Z2 Particle Counter and Size Analyzer, after the ^{67}Cu had decayed. The Cu-uptake rates ($\text{zmol} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1} = 10^{-21} \text{ mol} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1}$) for each Cu concentration were determined by linear regressions of the accumulation of Cu as a function of time and were normalized to cell surface area.

To determine the optimal duration for the ^{67}Cu -uptake experiments, initial experiments were performed for up to 6 h with Fe-replete *T. pseudonana* cultures. The results (data not shown) indicated that during the first 2 h, cellular Cu increased linearly. Thus, the sampling time for Cu-uptake experiments was limited to 2 h.

Cu-uptake kinetic parameters for cells grown under various Fe and/or Cu levels. Sixteen (2, 10, 30, 60, 80, 100, 120, 150, 180, 240, 300, 500, 700, 1,000, 1,500, and 2,000 nM; Fig. 1) or eight (2, 30, 60, 120, 240, 500, 1,000, and 2,000 nM; Fig. 2) Cu concentrations were used in the Cu-uptake kinetic experiments. For each growth treatment, two independent cultures were grown. Rates of Cu uptake for all Cu concentrations tested were measured for both duplicate cultures, from which a mean Cu-uptake rate ($\text{zmol} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1}$) was calculated. These mean Cu-uptake rates were used to calculate the V_{max} and K_m of each growth treatment, using the Michaelis-Menten equation (see Results) and the statistical program SigmaPlot 10.0 (Systat Software Inc. San Jose, CA, USA). The standard errors associated with the V_{max} and K_m parameters are the standard errors from the best-fit values. A *t*-test (TDIST) was used to compare the effect of Cu or Fe on various treatments. The

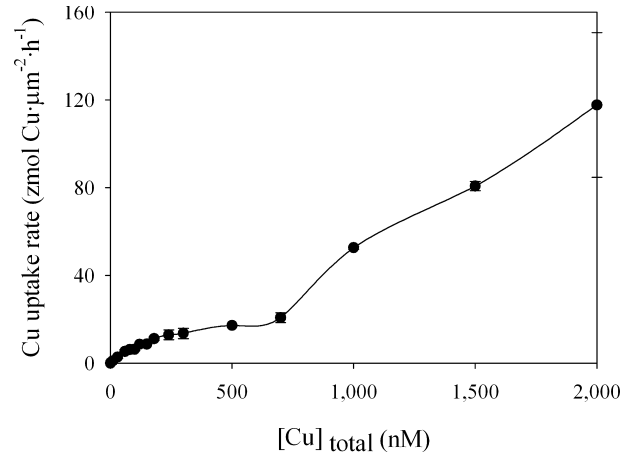


FIG. 1. Michaelis-Menten kinetics of Cu uptake as a function of total Cu concentrations in Fe- and Cu-replete *Thalassiosira pseudonana*. The rates of Cu uptake ($\text{zmol} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1} = 10^{-21} \text{ mol} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1}$) were determined as described in the Materials and Methods. The data points are the means of Cu-uptake rates of duplicate cultures, and the error bars represent the range. In these experiments, the EDTA concentration was kept constant at 100 μM , while the Cu concentrations were varied.

effects of Cu and Fe and its interaction (analysis of variance, ANOVA) were investigated only for the low-affinity Cu-transport system of *T. oceanica*, since this was the only transport system active in all Cu/Fe treatments.

RESULTS

Cellular characteristics. *T. oceanica* and *T. pseudonana* were acclimated to different Fe and Cu concentrations (Table 1). Fe limitation significantly decreased (ANOVA, $P < 0.001$) cell growth rate (d^{-1}), surface area (μm^2), and volume (fL). In contrast, the effects of Cu availability were only obvious (ANOVA, $P < 0.05$) on the cell surface area and volume of *T. oceanica* (Table 2) and showed a surprising increase with low Cu availability.

Kinetics of Cu transport. At Cu concentrations ranging from 2 to 500 nM, cellular Cu accumulation ($\text{zmol} \cdot \mu\text{m}^{-2}$) in *T. oceanica* and *T. pseudonana* increased linearly as a function of time in the course of 2 h (data not shown). Sixteen Cu concentrations (2, 10, 30, 60, 80, 100, 120, 150, 180, 240, 300, 500, 700, 1,000, 1,500, and 2,000 nM) were first

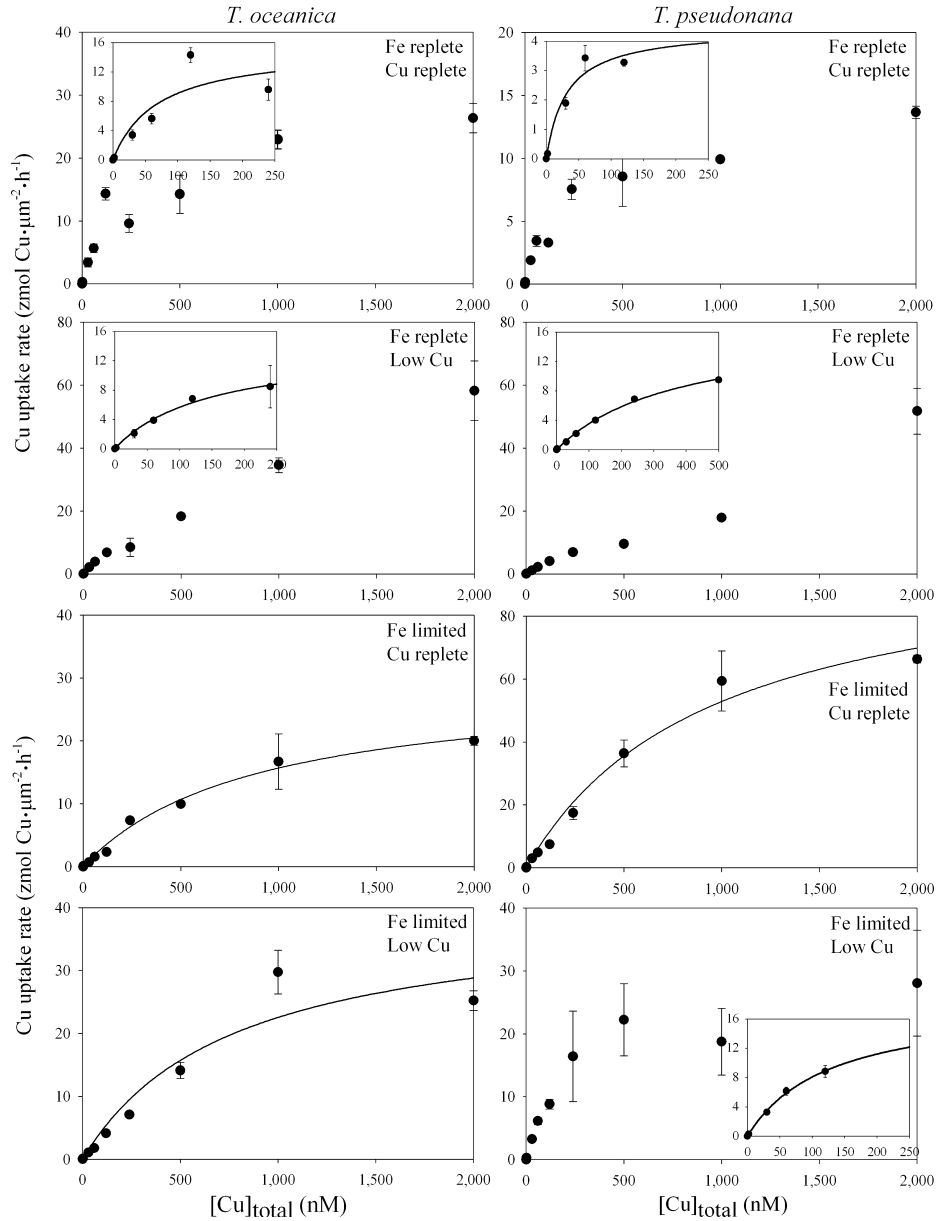


FIG. 2. Michaelis–Menten kinetics of Cu uptake as a function of total Cu concentrations in *Thalassiosira oceanica* and *Thalassiosira pseudonana* cultured under different Cu and Fe levels (see Table 1). The rates of Cu uptake ($\text{z mol} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1} = 10^{-21} \text{ mol} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1}$) were determined as described in the Materials and Methods. The data points are the means of Cu-uptake rates of duplicate cultures, and curves are the line of best fit for the double rectangular hyperbola equation. In these experiments, the EDTA concentration was kept constant at 100 μM , while the Cu concentrations were varied.

TABLE 2. Cellular characteristics (mean \pm SD) of live *Thalassiosira oceanica* and *Thalassiosira pseudonana* grown under different Fe and Cu concentration and pMe in each medium, see Table 1. ($\text{fL} = \mu\text{m}^3 = 10^{-15} \text{ L}$; n , the number of replicates).

	Treatment	Growth rate (d^{-1}) (n)	Cell surface area (μm^2)	Cell volume (fL)
<i>T. oceanica</i>	Fe replete/Cu replete	0.80 ± 0.09 (9)	85.62 ± 0.02	74.49 ± 0.03
	Fe replete/low Cu	0.73 ± 0.10 (9)	139.31 ± 0.36	154.61 ± 0.59
	Fe limited/Cu replete	0.51 ± 0.10 (9)	59.37 ± 0.67	43.01 ± 0.73
	Fe limited/low Cu	0.48 ± 0.08 (9)	70.60 ± 0.74	55.78 ± 0.88
<i>T. pseudonana</i>	Fe replete/Cu replete	1.79 ± 0.14 (8)	68.80 ± 1.30	53.67 ± 1.47
	Fe replete/low Cu	1.75 ± 0.15 (5)	70.29 ± 0.00	55.41 ± 0.00
	Fe limited/Cu replete	0.82 ± 0.09 (9)	41.85 ± 0.40	25.46 ± 0.34
	Fe limited/low Cu	0.73 ± 0.18 (5)	48.23 ± 1.30	31.50 ± 1.22

used in the Cu-uptake kinetic experiments. For these initial experiments, *T. pseudonana* grown under Fe- and Cu-replete condition was used as a

model. The Cu-uptake rates ($\text{z mol} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1}$) were a function of Cu concentrations and followed Michaelis–Menten uptake kinetics, approaching

saturation at ~250 nM Cu (Fig. 1). Interestingly, the Cu-uptake kinetics at these Cu concentrations clearly indicated that Cu uptake is biphasic (Fig. 1). Considering the limitation of the radioactivity of ^{67}Cu we can manipulate, in the rest of the Cu-uptake kinetic experiments, only eight Cu concentrations (2, 30, 60, 120, 240, 500, 1,000, and 2,000 nM) were chosen. Based on the statistical analysis, five out of eight treatments (graphs with inserts, Fig. 2) also exhibited biphasic Michaelis–Menten kinetics of Cu uptake as a function of Cu concentration (Fig. 2, Table 3). The first phase was observed at low Cu concentrations (~0–500 nM, see inserts), while the second phase was observed at higher Cu concentrations (up to 2,000 nM). This biphasic uptake saturation kinetic pattern was assumed to reflect the simultaneous operation of a high-affinity and a low-affinity uptake system at low and high Cu concentrations, respectively (Knauer et al. 1997). Thus, a biphasic Cu-uptake kinetic curve was fitted to our data using a double rectangular hyperbola equation (Nissen 1991) (Table 3):

$$[V = V_{\max 1}[\text{Cu}]_{\text{total}}/([\text{Cu}]_{\text{total}} + K_{m1}) + V_{\max 2}[\text{Cu}]_{\text{total}}/([\text{Cu}]_{\text{total}} + K_{m2})] \quad (1)$$

where V is the total Cu-uptake rate ($\text{zmol Cu} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1}$), $V_{\max 1}$ and $V_{\max 2}$ are the maximum uptake rates of the two uptake systems, $[\text{Cu}]_{\text{total}}$ is the total copper concentration, and K_{m1} and K_{m2} are the half-saturation constants of the two uptake systems. According to these analyses (Table 3), the K_m values were 50-, 870-, and 21-fold lower for the high-affinity Cu-transport system compared to the low-affinity Cu-transport system in Fe/Cu-replete, Fe-replete/low-Cu *T. oceanica* and Fe-limited/low-Cu *T. pseudonana*, respectively. For these same cultures, the V_{\max} values were also lower by 3.3-, 183- and 13-fold, respectively, for the high-affinity Cu-transport system. Fe/Cu-replete and Fe-replete/low-Cu *T. pseudonana*

also exhibited two Cu-transport systems. However, the low-affinity uptake system did not saturate, and as a result, the data could not be fitted by the double rectangular hyperbola equation. In addition, the K_m values of the high- and low-transport systems were identical in some cases (i.e., Fe-limited/Cu-replete and Fe-limited/low-Cu *T. oceanica*, and Fe-limited/Cu-replete *T. pseudonana*), so we assumed that only one transport system was active. Given the high K_m values (~1,000 nM), we classified these Cu-transport systems as low affinity.

Low-affinity Cu-uptake kinetics. *T. oceanica* and *T. pseudonana* had significantly different low-affinity Cu-uptake kinetics under Fe-replete conditions, but similar kinetics under Fe limitation (Fig. 2, Table 3). When Fe was sufficient, Cu uptake by *T. pseudonana* did not saturate. In contrast, Fe-sufficient *T. oceanica* exhibited saturating uptake kinetics. When Fe was limiting, both *T. oceanica* and *T. pseudonana* had similar K_{m2} (~1 μM) and V_{\max} (30 and 100 $\text{zmol Cu} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1}$, respectively). These results indicate that the low-affinity Cu-transport system of *T. oceanica* and *T. pseudonana* had different responses to Fe availability. Statistical analyses ($P < 0.05$, ANOVA and TDIST) indicate that Cu availability in the growth media had no significant effect on the K_m of the low-affinity Cu-transport system in *T. oceanica* and *T. pseudonana* (Table 4). Meanwhile, low Cu availability had a significant effect on the V_{\max} of low-affinity Cu uptake by *T. oceanica* ($P = 0.012$, ANOVA, Table 4) when Fe availability was high (6.5-fold faster for low-Cu cultures) and by *T. pseudonana* ($P = 0.007$, TDIST, Table 4) when Fe was limiting (2-fold slower for cultures grown under low Cu concentration). Fe limitation significantly affected only the V_{\max} of low-affinity Cu transport in *T. oceanica* grown under low Cu availability ($P = 0.015$, ANOVA, Table 4), where the V_{\max} of Fe-limited culture was 6-fold slower than that of the Fe-replete cultures. Fe also

TABLE 3. Kinetic parameters of the high- and low-affinity Cu-transport system in *Thalassiosira oceanica* and *Thalassiosira pseudonana* grown under various Fe and Cu levels. The K_m and V_{\max} values (\pm standard error of best-fit value) were calculated for the mean Cu-uptake rates of duplicate independent cultures measured at Cu concentrations ranging from 0 to 2,000 nM. The high- and low-affinity uptake system parameters were calculated using the double Michaelis–Menten equation $[V = V_{\max 1} [\text{Cu}]_{\text{total}}/([\text{Cu}]_{\text{total}} + K_{m1}) + V_{\max 2} [\text{Cu}]_{\text{total}}/([\text{Cu}]_{\text{total}} + K_{m2})]$. R^2 is the regression coefficient of the Michaelis–Menten equation fit to the data. For specific Fe and Cu concentration and pMe in each medium, see Table 1.

Treatment	Low affinity		High affinity		Cu range (nM)	R^2	
	K_m (nM)	V_{\max} ($\text{zmol Cu} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1}$)	K_m (nM)	V_{\max} ($\text{zmol Cu} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1}$)			
<i>T. oceanica</i>	Fe replete/Cu replete	2,410 \pm 8,335	36 \pm 48	48 \pm 110	11 \pm 13	0–2,000	0.942
	Fe replete/low Cu	6,336 \pm 2,253	238 \pm 63	7.3 \pm 32	1.3 \pm 1.1	0–2,000	0.998
	Fe limited/Cu replete	879 \pm 156	29 \pm 2.4			0–2,000	0.990
	Fe limited/low Cu	771 \pm 383	40 \pm 8.9			0–2,000	0.925
<i>T. pseudonana</i>	Fe replete/Cu replete	NA, did not saturate at 2,000 nM		188 \pm 48	12 \pm 1.1	0–1,000	0.971
	Fe replete/low Cu	NA, did not saturate at 1,000 and 2,000 nM		373 \pm 41	17 \pm 1.0	0–500	0.997
	Fe limited/Cu replete	923 \pm 222	101 \pm 11			0–2,000	0.982
	Fe limited/low Cu	1,117 \pm 525	63 \pm 4.9	52 \pm 129	4.8 \pm 7.8	0–2,000	0.997

TABLE 4. Effects (*P*-value, TDIST and two-way analysis of variance [ANOVA]) of Cu availability, Fe availability, and the interaction between Fe and Cu availability on K_m and V_{max} of the low- and high-affinity Cu-transport system in *Thalassiosira oceanica* and *Thalassiosira pseudonana*. For specific Fe and Cu concentration and pMe in each medium, see Table 1.

	Species	Treatment	Kinetic parameter	Metal effect	<i>P</i> -value	Statistics	
High affinity	<i>T. pseudonana</i>	Fe replete/Cu replete vs. Fe replete/low Cu	K_m	Cu	0.010	TDIST	
			V_{max}	Cu	0.005		
Low affinity	<i>T. pseudonana</i>	Fe replete/low Cu vs. Fe limited/low Cu	K_m	Fe	0.031	TDIST	
		Fe limited/Cu replete vs. Fe limited/low Cu	V_{max}	Cu	0.007	TDIST	
	<i>T. oceanica</i>	All treatments		V_{max}	Fe	0.015	Two-way
					Cu	0.012	ANOVA
				Fe and Cu	0.023		

had a significant effect on the low-affinity Cu-transport system in *T. pseudonana* (Table 3), although we were unable to obtain robust fits to the data to compare the K_m and V_{max} values statistically. The Cu uptake in Fe-replete *T. pseudonana* was not saturated at 1,000 or 2,000 nM Cu compared to Fe-limited treatments.

High-affinity Cu-uptake kinetics. *T. oceanica* and *T. pseudonana* had different K_m and V_{max} kinetics for the high-affinity Cu-transport system depending on the Fe and Cu availability (Fig. 2, Table 3). When Fe and Cu were sufficient, both of these diatoms had similar K_m (~50–200 nM) and V_{max} (~10–12 $\mu\text{mol} \cdot \mu\text{m}^{-2} \cdot \text{h}^{-1}$). For cultures grown under low Cu, *T. oceanica* had a lower half-saturation constant (51-fold lower K_m , $P < 0.0001$, TDIST) and a slower maximum rate of Cu uptake (13-fold lower V_{max} , $P < 0.0001$, TDIST) than *T. pseudonana*. Moreover, when Fe was limiting, the high-affinity Cu-transport system seemed to disappear in *T. oceanica* and in Cu-replete *T. pseudonana*. Within the error of the estimates (Table 4), Cu availability had no significant effect on the K_m and V_{max} of high-affinity Cu-transport system in *T. oceanica*. However, low Cu significantly increased K_m and V_{max} ($P = 0.010$ and 0.005) in Fe-replete *T. pseudonana* (Fe replete/low Cu vs. Fe replete/Cu replete, Table 4). Fe limitation had significant effects on the high-affinity Cu-uptake kinetics of both species (Table 4). When Fe concentration was low, the high-affinity Cu-transport system in *T. oceanica* was down-regulated (turned off). Similarly, Fe limitation turned off the high-affinity transport system of *T. pseudonana* when Cu was replete. In contrast, Fe limitation up-regulated the high affinity of *T. pseudonana* when Cu was low by decreasing its K_m by ~7-fold ($P = 0.031$, Table 4).

DISCUSSION

Cu-uptake kinetics of phytoplankton. The Michaelis-Menten kinetics of Cu uptake by the centric diatoms *T. oceanica* and *T. pseudonana* seem to follow biphasic kinetics when cells are exposed to total Cu concentrations ranging from 2 to 2,000 nM. Biphasic uptake kinetics are usually ascribed to the presence of two uptake systems, a high- and a low-affinity uptake system operating simultaneously (Epstein

et al. 1963, Komor and Tanner 1975). These uptake kinetics have been reported in many organisms for various substrates, including sugar transport in the green alga *Chlorella* (Komor and Tanner 1975), nitrate uptake by marine phytoplankton (Collos et al. 1997), glucose transport by fungi (Schneide and Wiley 1971), and Cu and phosphate uptake by higher plants (Austenfeld and Veltrup 1983, Perez-Llorens and Niell 1995). For Cu transport, two uptake systems have been found in the freshwater green alga *Scenedesmus subspicatus* (Knauer et al. 1997), where the K_m of the low-affinity and high-affinity Cu-transport systems are 2.82 pM and 0.07 pM of free Cu^{2+} , respectively. The research herein demonstrated that the K_m values of the low- and high-affinity Cu-transport system for *T. oceanica* grown under Fe/Cu-sufficient conditions were 2,410 nM and 48 nM total Cu, respectively, thus differing by 50-fold. If these K_m values, expressed as total Cu concentrations, are converted to free Cu^{2+} concentrations (using the side reaction coefficient and conditional thermodynamic constant of CuEDTA reported in Sunda et al. 2005), the resulting K_m values are 1.84 pM and 0.04 pM, respectively. These K_m values are very similar to those values of *S. subspicatus*. The K_m value of the high-affinity Cu-transport system in *T. pseudonana* is also low, 188 nM (0.14 pM expressed in free Cu^{2+}), but 3.5- and 2-fold higher than the K_m of *T. oceanica* and *S. subspicatus*, respectively. The Cu-transport kinetics of the diatoms in this study are also different from that of the green alga *C. reinhardtii* (Hill et al. 1996). In *C. reinhardtii*, only one temperature-dependent Cu-transport system has been found, with a K_m of ~210 nM total Cu (Hill et al. 1996). Furthermore, Michaelis-Menten Cu-uptake kinetics have been determined for a limited set of marine phytoplankton by Croot et al. (2003). A similar Cu-transport pathway exists in a cyanobacterium and a dinoflagellate. The K_m values of the cyanobacteria *Synechococcus* sp. DC2 and the dinoflagellate *Amphidinium carterae* were ~15.5 pM and 39.8 pM free Cu^{2+} , respectively. These high K_m values are characteristic of low-affinity Cu-transport systems. However, it is important to note that Croot et al. (2003) did not conduct the Cu-uptake experiments with cultures grown at the low Cu concentration used in our study and that of

Knauer et al. (1997). Assuming that the K_m reported by Croot et al. (2003) are those of a low-affinity Cu-transport system, the low-affinity Cu-transport system of our diatom *T. oceanica* has higher affinity for Cu (8- or 22-fold lower K_m) than those of *Synechococcus* and *Amphidinium*.

The V_{max} of low- and high-affinity Cu-transport systems are also normally different. In general, the low K_m of a high-affinity transport system results in a slower V_{max} (Raymont 1980, Kajikawa et al. 1997, Persson et al. 2000). In *S. subspicatus*, the V_{max} of high-affinity Cu-transport system is 28-fold lower than that of the low-affinity Cu-transport system (Knauer et al. 1997). In the diatoms *T. oceanica* and *T. pseudonana*, the mean difference between V_{max} of the high-affinity and low-affinity transport system is ~90-fold but ranges from 3- to 182-fold.

Quigg et al. (2006) measured the short-term Cu accumulation rate by *T. pseudonana* in natural seawater (Sandy Hook seawater) with 28 pM Cu'. Their Cu-uptake rate, 546 zmol Cu · μm^{-2} · h⁻¹ (9.1 zmol Cu · μm^{-2} · min⁻¹, Quigg et al. 2006), is higher than that reported here (13.7 zmol Cu · μm^{-2} · h⁻¹) for Fe/Cu-replete *T. pseudonana* at 2,000 nM total Cu (with 25 pM Cu'). This 40-fold difference may be due to a disparity in intracellular Fe and Cu concentrations between the *T. pseudonana* cultures and/or the sampling time for the determination of Cu-uptake rates. In Quigg et al. (2006), *T. pseudonana* was acclimated in AQUIL medium with 84 nM dissolved Fe, whereas we used 1,370 nM dissolved Fe for the Fe-replete culture and 12.5 nM for the Fe-limited ones. In addition, while our sampling time was 2 h, that of Quigg et al. was only 20 min. Indeed, if we calculate Cu-uptake rates for only the first time point (15 min), our Cu-uptake rate of the Fe-limited/Cu-replete *T. pseudonana* (537 zmol Cu · μm^{-2} · h⁻¹, measured at 2,000 nM total Cu, or 25 pM Cu') is almost identical to that measured by Quigg et al. (546 zmol Cu · μm^{-2} · h⁻¹).

T. oceanica versus *T. pseudonana* Cu-uptake kinetics. Although the data presented here suggest that *T. oceanica* and *T. pseudonana* have both a low- and a high-affinity Cu-transport system, there were distinct differences between these two species. For example, the low-affinity transport systems for *T. oceanica* and *T. pseudonana* grown under Fe/Cu-replete conditions were very different: while that of *T. oceanica* saturated at ~2,000 nM, that of *T. pseudonana* showed no saturation at that Cu concentration. These results suggest that under Fe/Cu-replete conditions, *T. pseudonana*, the coastal isolate, has more-efficient intracellular Cu homeostasis mechanisms than *T. oceanica*. These mechanisms may include Cu efflux and/or intracellular metal-binding peptides. Indeed, some freshwater and marine phytoplankton have been documented to efflux internalized Cu (Verma and Singh 1991, Knauer et al. 1997, Pandey et al. 1997, Croot et al. 2003). Cu storage by phytochelatins has been observed in marine phyto-

plankton (Ahner et al. 1994, 1997, 2002, Morelli and Scarano 2004).

The low-affinity Cu-uptake systems of *T. oceanica* and *T. pseudonana* were similar ($K_m \sim 1 \mu\text{M}$ and V_{max} 30–100 zmol Cu · μm^{-2} · h⁻¹) only when cells were Fe limited. Interestingly, Fe limitation down-regulated (or turned off) the high-affinity Cu-transport system in both diatoms. In the case of *T. oceanica*, the high-affinity Cu-transport system seemed to be turned off by Fe limitation in both Cu growth conditions (Cu replete and low Cu), while that of *T. pseudonana* was turned off by Fe limitation only under Cu-replete conditions. In contrast, Fe-limited *T. pseudonana* grown with low Cu up-regulated its high-affinity Cu-transport system. These changes may reflect the need to take up extra Cu when Fe is low due to the up-regulation of multicopper oxidase in the high-affinity Fe transport system. Therefore, Fe nutrition controls Cu uptake differently in *T. pseudonana* and *T. oceanica*. In addition, when Fe-sufficient cells were subjected to low Cu levels, the high-affinity Cu-transport system of *T. oceanica* had significantly lower K_m (51-fold, $P < 0.0001$, TDIST) and V_{max} (13-fold, $P < 0.0001$, TDIST) than that of *T. pseudonana*. Thus, the high-affinity Cu-transport system of Fe-replete *T. oceanica* is more efficient under low Cu than that of *T. pseudonana*. This potentially reflects the lower Cu concentrations in the open ocean and the evolution of a diatom in a chronically low-Fe environment, where intracellular Cu may have partially relieved Fe use in some key biochemical pathways.

Strong Cu and Fe effects on Cu-uptake kinetics. Cu and Fe availabilities in growth media have a strong effect on the Cu-uptake kinetics of *T. oceanica* and *T. pseudonana*, and an interaction between Fe and Cu exists in these diatoms. However, the results obtained here indicate that Cu-uptake kinetics are complex and suggest that Cu transport is regulated dynamically by both Fe and Cu. At present, we are unable to explain all the interactions between Cu and Fe nutrition on Cu transport. Thus, we have restricted this discussion to the most significant results.

Cu availability had significant effects on the V_{max} of low-affinity Cu uptake in *T. oceanica* ($P = 0.012$, two-way ANOVA, Table 4) and *T. pseudonana* ($P = 0.007$, TDIST, Table 4), and these effects were different depending on Fe availability ($P = 0.023$, two-way ANOVA, Table 4). When Fe is sufficient, the V_{max} of low-affinity Cu transporter in *T. oceanica* is up-regulated under low Cu (6.5-fold higher for low-Cu cultures, $P = 0.021$, TDIST). Similar up-regulation of the low-affinity Cu-transport system has been observed in *C. reinhardtii* (Hill et al. 1996), where the V_{max} in Cu-deficient cells ($169 \pm 9 \text{ amol} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$) is 20-fold faster than that of Cu-sufficient cells ($8.16 \pm 0.6 \text{ amol} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$). Thus, in *C. reinhardtii*, the same Cu-transport system operates in Cu-replete and Cu-deficient cells, but more

Cu transporters are expressed under Cu-deficient conditions (Hill et al. 1996). In a similar manner, Fe-limited *T. oceanica* may be expressing a higher density of low-affinity Cu transporters when Cu is low. In contrast to this finding, low Cu in Fe-limited *T. pseudonana* elicited slower V_{\max} ($P = 0.007$, TDIST, Table 4). As for the effects of Fe availability on Cu-uptake kinetics, it has been covered in the section discussing *T. oceanica* versus *T. pseudonana*.

High-affinity and low-affinity Cu transporters in eukaryotes. In the yeast *S. cerevisiae*, Cu transport is mediated by a high- and a low-affinity uptake system depending on Cu availability. In the high-affinity transport system, Cu is reduced from Cu^{2+} to Cu^+ by plasma membrane reductases, including highly specific cupric reductases (Hassett and Kosman 1995) and FRE1, which also functions in Fe transport (Georgatsou et al. 1997). After Cu is reduced, Cu^+ is taken up by Cu-transport proteins (Ctr1p and Ctr3p), which are localized in the plasma membrane (Dancis et al. 1994b, Labbe et al. 1997, Pena et al. 2000). The expression of these proteins is regulated by intracellular Cu deficiency. In phytoplankton, it has been demonstrated that cupric reductase activity exists at the cell surface (Jones et al. 1987, Hill et al. 1996). Recently, homologs (*Tp*FRE1-3) to these reductases of the high-affinity Cu and Fe transport system in yeast (FRE1 AND FRE2) were identified in the genome of the diatom *T. pseudonana* (Kustka et al. 2007). These reductases thus may also have a cupric reductase function in the high-affinity Cu-transport system in *T. pseudonana*. CTR, a Cu permease, is deemed to be a canonical member of a high-affinity Cu transport family of proteins in eukaryotic organisms (Puig and Thiele 2002, Page et al. 2009). A putative CTR has also been found in the genome of *T. pseudonana* (Thaps3/chr_11a:66327-68695, see <http://genome.jgi-psf.org/Thaps3/Thaps3.home.html>). This transporter may be the high-affinity Cu-uptake transporter of marine diatoms.

Potential transporters in the low-affinity Cu-transport system include FET4 (also a low-affinity Fe^{2+} permease) and SMF1 in yeast (Dix et al. 1997, Liu et al. 1997, Hassett et al. 2000). In phytoplankton, P-type ATPase Cu^{2+} transporters are found in the thylakoid membrane and in the plasma membrane of the freshwater *Synechococcus* species PCC7942 (Kanamaru et al. 1994, Phung et al. 1994). Cu-transporting ATPases exist in the genome of the marine diatom *T. pseudonana* (thaps1_ua_kg.chr_16a000004, see <http://genome.jgi-psf.org/Thaps3/Thaps3.home.html>) and *Synechococcus* WH8102 (Palenik et al. 2003). These P-type ATPases may regulate low-affinity Cu transport in marine phytoplankton. The low-affinity Cu transporters may also involve members of the NRAMP (Natural Resistance-Associated Macrophage Protein) and the ZIP/IRT (Zinc IRT-like Protein/Fe-Regulated Transporter) families due to their wide selectivity for cations (Cu^{2+} , Cd^{2+} , Fe^{2+} , Mn^{2+} , and Zn^{2+})

(Gunshin et al. 1997, Grotz et al. 1998, Korshunova et al. 1999, Ramesh et al. 2003). Indeed, physiological studies of marine phytoplankton have indicated that high availability of Cu^{2+} inhibits cellular Mn and Zn uptake in the diatom *T. pseudonana* (Sunda and Huntsman 1983, 1996), supporting a common transporter for Cu, Mn, and Zn. We thus propose that in marine diatoms, different, nonspecific metal transporters may work together as low-affinity Cu transporters.

Oceanographic implications. Using diatoms as model organisms, this study presents the first evidence of the co-occurrence of a high- and a low-affinity Cu-transport system in marine phytoplankton. One of our principal observations from this work is that the characteristics of these Cu-transport systems are different in the coastal and the oceanic diatoms investigated here, potentially reflecting the evolutionary adaptation of species to different trace-metal regimes. The open-ocean isolate, *T. oceanica*, has the highest affinity for Cu transport of any phytoplankton tested to date, which is consistent with the very low Cu concentrations in offshore oceanic waters. In contrast, the coastal strain, *T. pseudonana*, was able to tolerate relatively higher Cu concentrations that are typical for coastal waters. Moreover, the Cu-uptake systems in the two diatom species are differentially controlled by Cu and/or Fe nutrition. For example, while Fe-replete *T. oceanica* lowered the K_m and V_{\max} of its high-affinity Cu-transport system under low-Cu conditions, such a response is not observed in *T. pseudonana*. Another disparity between these two strains is their ability to increase the number of Cu transporters at the cell surface (V_{\max}) in response to low Cu. As observed in other phytoplankton (Hill et al. 1996), *T. oceanica* increased the V_{\max} of the low-affinity Cu-transport system in response to low Cu, while *T. pseudonana* decreased its V_{\max} . However, the responses of Cu-uptake kinetics to different Fe and/or Cu conditions are complex, providing more support for the dynamic interaction between Fe and Cu in diatom physiology and highlighting the need for further study.

The Cu-uptake rates determined here using CuEDTA, a nonphotolabile organic Cu complex (Natarajan and Endicott 1973), question our present understanding of what controls Cu transport in marine phytoplankton. The free-ion model (Hudson 2005) assumes that only inorganic metal species are labile and utilized directly by phytoplankton, while organically bound metals are essentially unavailable. However, using CuEDTA as a model organic Cu complex, our results suggest that the flux of inorganic Cu to the cell surface (J_D) is insufficient to account for the Cu-uptake rates we measured (ρCu). When both diatoms were grown in Fe/Cu-replete conditions, the observed Cu-uptake rates, at six different Cu concentrations ranging from 2 to 500 nM, exceeded the corresponding

TABLE 5. Measured Cu-uptake rates (ρCu) by *Thalassiosira oceanica* and *Thalassiosira pseudonana* grown in Fe/Cu-replete media, maximum diffusive rate of dissolved inorganic Cu (Cu') from the bulk solution to the surface of the diatom cell (J_D), and the ratio of $\rho\text{Cu}:J_D$. The measured Cu-uptake rates reported here are the average linear uptake rates (\pm range) of two independent cultures. The maximum diffusive rate of Cu' was calculated using the equation $J_D = 4\pi rD[\text{Cu}']$, where r (cm) is the cell radius and D ($2.16 \times 10^{-2} \text{ cm}^2 \cdot \text{h}^{-1}$ at 20°C) is the diffusion coefficient of dissolved inorganic Cu (Hudson and Morel 1993). The concentration of free Cu^{2+} (ρCu) was calculated using the program MINEQL + 4.61 (Environmental Research Software). The concentration of Cu' was calculated from the inorganic side reaction coefficient ($\alpha_{\text{Cu}} = [\text{Cu}']/[\text{Cu}^{2+}] = 16.1$, Sunda et al. 2005).

[Cu] _{total} (nM)	[Cu'] (pM)	ρCu	Measured Cu-uptake rate (ρCu) ($\text{zmol Cu} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$)		Max Cu' diffusion rate (J_D) ($\text{zmol Cu} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$)		Ratio ($\rho\text{Cu}:J_D$)	
			<i>T. oceanica</i>	<i>T. pseudonana</i>	<i>T. oceanica</i>	<i>T. pseudonana</i>	<i>T. oceanica</i>	<i>T. pseudonana</i>
2	0.02	14.8	26 \pm 8	11 \pm 0.5	1.7	1.6	15.1	7.3
30	0.4	13.6	291 \pm 59	130 \pm 13	26	23	11.1	5.6
60	0.7	13.3	482 \pm 60	235 \pm 29	52	47	9.2	5.0
120	1.5	13.0	1,224 \pm 84	225 \pm 8	105	94	11.7	2.4
240	2.9	12.7	820 \pm 123	518 \pm 55	209	188	3.9	2.8
500	6.2	12.4	1,220 \pm 260	587 \pm 161	436	391	2.8	1.5
1,000	12	12.1	1,965 \pm 127	681 \pm 1	872	781	2.3	0.9
2,000	25	11.8	2,256 \pm 199	939 \pm 34	1,743	1,563	1.3	0.6

calculated maximum diffusion rates of inorganic Cu by factors of 1.5 to 15.1 (Table 5). The mean ratio of $\rho\text{Cu}:J_D$ was 7.2 and 3.3 for *T. oceanica* and *T. pseudonana*, respectively. For only *T. pseudonana*, at the two highest Cu concentrations (1,000 nM and 2,000 nM), the diffusive flux of inorganic Cu fully accounted for the Cu-uptake rates measured. Faster rates of Cu uptake relative to the diffusive flux of inorganic Cu to the cell surface has also been observed in recent studies (Quigg et al. 2006, Annett et al. 2008). These results suggest that diatoms might be able to access Cu bound within organic Cu complexes. Thus, Cu uptake may not be always controlled by the free Cu^{2+} or the Cu' concentration, but instead by total Cu concentration. Further study is needed to investigate this Cu-uptake mechanism. Reductive release of Cu from organically bound Cu^{2+} complexes by cell surface cupric reductases may be one mechanism of Cu uptake in marine diatoms (Semeniuk et al. 2009). In addition, at identical Cu' concentrations, the ratios of Cu uptake to maximum diffusive fluxes of inorganic Cu were 1.4 to 5 times higher in *T. oceanica* than in *T. pseudonana*. *T. oceanica* may thus have higher Cu(II) reductive ability or higher affinity Cu^+ transporters, which is supported by the lower K_m of the high-affinity transport system of *T. oceanica* (48 nM) relative to *T. pseudonana* (188 nM).

Our field investigations in Station Papa in the subarctic Pacific in September of 2006 and May 2008 provide further evidence of the availability of organic Cu to indigenous phytoplankton. At an in situ Cu concentration of 2 nM, we measured ρCu of 2.3 and 5.8 $\mu\text{mol Cu} \cdot \text{mol C}^{-1} \cdot \text{h}^{-1}$, respectively (Semeniuk et al. 2009, D. Semeniuk and M. Maldonado, unpubl. data). Assuming that the mean phytoplankton size is $\sim 3 \mu\text{m}$ in radius and that 0.4% of total Cu is inorganic Cu (Donat and Bruland 1995), we calculate a diffusive flux of Cu on the order of 0.5 $\mu\text{mol Cu} \cdot \text{mol C}^{-1} \cdot \text{h}^{-1}$. The rates of Cu uptake by indigenous phytoplankton are thus five to 10

times faster than the inorganic Cu diffusive flux, indicating that Cu uptake in the open ocean might be controlled by the total Cu concentration. Given the K_m s of the low- and high-affinity Cu-transport system for our oceanic isolate (2,410 nM and 48 nM, respectively), indigenous phytoplankton most likely have an active high-affinity Cu-transport system in the open ocean, where total Cu concentration is ~ 2 nM (Bruland and Franks 1983, Rutgers van der Loeff et al. 1997, Moffett and Dupont 2007). Our study raises fundamental questions about Cu availability in the world's oceans. It also highlights the effects that phytoplankton Cu acquisition mechanisms, which potentially involve cupric reductases, may have on the distribution and speciation of Cu in seawater. The link between Fe and Cu nutrition is also evident, but further studies are needed to fully elucidate the complex interaction between these two metals in phytoplankton physiology.

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