

## Effect of pH on measurement of strong copper binding ligands in lakes

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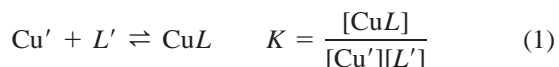
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### Abstract

We investigated the effect of pH on Cu<sup>2+</sup> binding by natural organic ligands in two New Zealand lakes on the South Island, New Zealand, using competitive ligand equilibration with salicylaldoxime and detection by cathodic stripping voltammetry. When the pH of a lake-water sample was adjusted to different values in the range 6.3–8.0, the conditional Cu<sup>2+</sup> binding constant  $K''$  was found to increase, with a slope log  $K''$  versus pH of nearly +2, as would be expected for functional groups having a proton dissociation constant  $pK_a > 9$ . In support of this, a comparison of log  $K''$  values measured on samples taken from surface and subsurface waters of two New Zealand alpine lakes over a 2-yr period showed a very similar log  $K''$ -pH dependence. These results imply that the functional group chemistry of strong Cu<sup>2+</sup>-binding ligands in such lakes is relatively uniform and may involve phenolic OH groups. In Lake Hayes, the Cu-binding ligand concentration  $[L]_T$  exceeded that of total dissolved Cu,  $[Cu]_T$  at almost all times of the year and all depths. However, in Lake Manapouri, little evidence of Cu-binding ligand was observed during late summer in the mixed layer, which suggests a seasonal cycle in Cu-binding ligands that is perhaps driven by enhanced ultraviolet irradiation in summer or by seasonal changes in phytoplankton community structure. This may have important consequences for the toxicity of Cu<sup>2+</sup> to organisms in these lakes during summer.

In recent years, it has become increasingly clear that the chemical speciation of several trace metals (e.g., Cu) in some aquatic systems is controlled by the formation of strong complexes with natural organic matter (NOM) ligands of apparent biological origin (Bruland et al. 1991). These ligands act to significantly reduce the concentration of the free metal ion, the biologically active form (Sunda and Guillard 1976; Coale and Bruland 1988; Moffett et al. 1990; Donat et al. 1994; Kozelka and Bruland 1998). In marine systems, the production of such bioligands by particular phytoplankton species is now well established (Moffett et al. 1990; Moffett and Brand 1996). Thus, an emerging new paradigm is that phytoplankton may actively control the in vitro biological availability of metals that are essential for biochemical functions and also those that may interfere with these functions. Much less is known about NOM ligands in freshwater systems; however, the existence of strong Cu-binding ligands has been reported in several Swiss lakes and rivers (Xue and Sigg 1993; Xue et al. 1995, 1996; Xue and Sunda 1997) and a productive seasonally anoxic English lake (Achterberg et al. 1997).

In marine systems, the binding of Cu<sup>2+</sup> by an organic ligand  $L$  is usually formulated in the following simplified manner (Bruland et al. 1991)



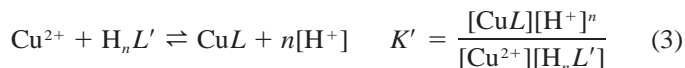
$[Cu']$  is the concentration of all Cu<sup>2+</sup> species composed of either the free Cu<sup>2+</sup> aquo ion or kinetically labile complexes formed with simple inorganic (e.g., OH<sup>-</sup>, Cl<sup>-</sup>, SO<sub>4</sub><sup>2+</sup>, or CO<sub>3</sub><sup>2-</sup>) or organic ligands (Cu' is often termed labile Cu). Similarly,  $[L']$  is the concentration of the ligand  $L$  not bound

to metal ions such as Cu<sup>2+</sup>. Thus the formation constant  $K$  is a conditional equilibrium constant that implicitly contains terms that describe the side reactions of both Cu<sup>2+</sup> and  $L$ . In the case of Cu<sup>2+</sup>, the side reactions with simple ligands are generally known, and the relationship between the concentration of free Cu<sup>2+</sup> and  $[Cu']$  can be calculated from knowledge of the water composition and the appropriate formation constants for each complex:

$$\begin{aligned} [Cu'] &= [Cu^{2+}] + [CuOH^+] + [CuCl^+] + [CuCO_3^{2-}] \cdots \\ &= [Cu^{2+}](1 + \beta_{CuOH}[OH^-] + \beta_{CuCl}[Cl^-] \\ &\quad + \beta_{CuCO_3}[CuCO_3^{2-}] + \cdots) \\ &= [Cu^{2+}]\alpha_{Cu} \end{aligned} \quad (2)$$

where  $\alpha_{Cu}$  is the side reaction coefficient for the formation of labile Cu<sup>2+</sup> complexes. It should be noted that, because of the terms involving concentrations of OH<sup>-</sup> and CO<sub>3</sub><sup>2-</sup> in Eq. 2, the side reaction coefficient  $\alpha_{Cu}$  is not constant in freshwater systems but depends on both pH and the ionic composition of the water. Similarly, the side reactions for the ligand  $L$  are not usually known but will include competitive binding with at least H<sup>+</sup> and possibly other metal ions in the medium, such as Ca<sup>2+</sup> and Mg<sup>2+</sup>.

In seawater, both pH and the concentrations of competing inorganic ligands are almost constant, so that the simplifications introduced by Eq. 1 still allow comparisons between the measured values of  $K$  for Cu-ligand binding made by different workers or in different parts of the ocean. However, in freshwaters, both pH and the concentrations of competing inorganic ligands vary considerably between different locations and in both space and time within a given freshwater system. Thus, Eq. 1 is perhaps better written in the more general form



The use of the free Cu<sup>2+</sup> concentration in Eq. 3 takes explicit account of the fact that many of the competing side reactions

### Acknowledgments

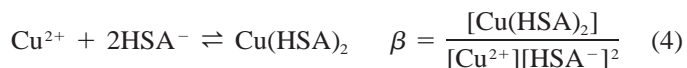
We thank Ken Bruland, Geoff Smith, Paul Meredith, Malcolm Reid, Sylvia Sander, and Carolyn Walker for help with this research.

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of metal ions with inorganic ligands (e.g.,  $\text{OH}^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{HCO}_3^-$ ) present in the water are themselves pH-dependent (Hunter et al. 1999). The number  $n$  of  $\text{H}^+$  released per  $\text{Cu}^{2+}$  taken up by the ligand  $L$  can only be determined experimentally. For a ligand that contains a single type of  $\text{Cu}^{2+}$ - and  $\text{H}^+$ -binding functional group, an integral value of  $n$  is expected (i.e.,  $n = 0, 1, 2, \dots$ ), depending on the stoichiometry of the  $\text{H}^+$ - $\text{Cu}^{2+}$  exchange reaction(s) and whether the solution pH is larger than the  $pK_a$  of the functional groups (complete  $\text{H}^+$  dissociation) or smaller (incomplete  $\text{H}^+$  dissociation). For molecular assemblages that contain a variety of different functional groups, some kind of average non-integral value would be expected. Also, for polymeric ligands bearing an electrical charge, both variations in the acidity of the ligand functional groups and electrostatic effects may lead to nonintegral values of  $n$  (Tanford 1961). A comparison of Eqs. 1 and 3 shows that the conditional constant  $K$  in Eq. 1 contains an implicit pH dependence.

It should be noted that Eq. 3 still contains implicit terms (embodied in  $K'$  and  $[L']$ ) for the effects of competing metal ions such as  $\text{Ca}^{2+}$  on the  $\text{Cu}^{2+}$ - $L$  equilibrium. Because variations in the concentrations of ions such as  $\text{Ca}^{2+}$  are generally much smaller than those of  $\text{H}^+$  in most freshwaters, we chose first to focus on the role of pH. Thus, we report on an experimental study of the effects of pH on  $\text{Cu}^{2+}$ - $L$  binding equilibria in two New Zealand lakes.

For the measurement of  $\text{Cu}^{2+}$  binding by NOM, we used the technique of competitive ligand equilibration (CLE) in which a competitive  $\text{Cu}^{2+}$ -binding equilibrium is set up between the NOM ligand  $L$  and a synthetic ligand of known properties. In our case, we used salicylaldoxime, a diprotic ligand ( $\text{H}_2\text{SA}$ ) for which the bis-complex formed between  $\text{Cu}^{2+}$  and the singly deprotonated species  $\text{HSA}^-$  dominates at  $\text{pH} > \sim 6.5$  (Kogut and Voelker, unpubl. data):



The advantage of this approach is that the species  $\text{Cu}(\text{HSA})_2$  adsorbs strongly at a mercury electrode, so that its in situ concentration can be measured by cathodic stripping voltammetry (CSV; Campos and Van Den Berg 1994). From this and a knowledge of the side reaction coefficient for the formation of the  $\text{Cu}(\text{HSA})_2$  complex through Eq. 4, the concentration of free  $\text{Cu}^{2+}$  can be directly calculated. In the situation where the ligand  $\text{H}_2\text{SA}$  (total concentration  $[\text{SA}]_T$ ) is present in excess relative to  $\text{Cu}^{2+}$ , the pH-dependent form of this side reaction coefficient derived from Eq. 4:

$$\begin{aligned} \alpha_{\text{Cu}(\text{HSA})_2} &= \frac{[\text{Cu}(\text{HSA})_2]}{[\text{Cu}^{2+}]} = \beta[\text{HSA}^-]^2 \\ &= \beta[\text{SA}]_T^2 \left( \frac{[\text{H}^+]}{K_1} + 1 + \frac{K_2}{[\text{H}^+]} \right)^{-2} \end{aligned} \quad (5)$$

where  $K_1$  and  $K_2$  are the first and second proton dissociation constants for  $\text{H}_2\text{SA}$ .

From Eq. 3, we can also define the corresponding side reaction coefficient for formation of  $\text{CuL}$ :

$$\alpha_{\text{CuL}} = \frac{[\text{CuL}]}{[\text{Cu}^{2+}]} = K' \frac{[L']}{[\text{H}^+]^n} \quad (6)$$

Finally, from Eqs. 2, 5, and 6, it follows that

$$[\text{Cu}]_T = [\text{Cu}^{2+}](\alpha_{\text{Cu}} + \alpha_{\text{CuL}} + \alpha_{\text{Cu}(\text{HSA})_2}) \quad (7)$$

Eq. 7 allows a pH-dependent description of  $\text{Cu}^{2+}$  binding by a single NOM ligand. We measured the effects of pH on  $\text{Cu}^{2+}$  binding by NOM in two different ways: by adjusting the pH of a lake water sample to different values and by taking advantage of natural pH variations over time and depth within two different lakes.

## Materials and methods

Surface and subsurface water samples were taken from two glacially formed alpine lakes in southern New Zealand—Lake Manapouri (440 m deep) and Lake Hayes (35 m deep). The trace metal and major ion geochemistry of these lakes have been recently described (Reid et al. 1999; Ellwood et al. 2001). Both lakes are vertically homogeneous in austral winter (July), developing in spring a strong thermocline centered on depths of 50 m in Lake Manapouri and 16 m in Lake Hayes. In Lake Manapouri, which does not become anoxic at depth, vertical profiles of most major ions and trace metals are essentially uniform throughout the year, with a mean dissolved  $[\text{Cu}]$  of 6.4 nmol  $\text{L}^{-1}$ . Lake Hayes is also vertically homogeneous outside summer months, with a mean dissolved  $[\text{Cu}]$  of 1.6 nmol  $\text{L}^{-1}$ . However, this lake develops an anoxic hypolimnion during summer, with high concentrations of dissolved Fe and Mn. In this region, the dissolved  $[\text{Cu}]$  falls below 1 nmol  $\text{L}^{-1}$  at depths  $>15$  m (Reid et al. 1999).

Samples for total dissolved Cu and for Cu speciation determinations were collected in a trace metal “clean” manner (Ahlers et al. 1990); 0.5 or 1-liter fluorinated polyethylene sample bottles were acid-cleaned and sealed in zip-lock plastic bags. Surface samples were collected by deployment of an acid-cleaned Teflon tube in front of the bow of an inflatable boat while moving forward into clean water. Water was pumped through the Teflon hose and tubing (C-flex) contained in the pump head of a portable peristaltic pump (Masterflex; Cole Palmer), next through a filtering cartridge (0.45  $\mu\text{m}$  pore size, polysulfone), and then directly into the sample bottle (Flegal et al. 1991). Water at depth was also collected in Teflon-coated, 2.5-liter Go-Flo bottles suspended on Kevlar hydroline and weighted with a conductivity-temperature-depth probe, to obtain temperature with depth. The Go-Flo bottles were triggered with solid PVC messengers. These samples were also filtered through a similar smaller cartridge (0.45  $\mu\text{m}$  pore size) immediately after collection. In Lake Hayes, subsurface samples were collected at 5, 10, 15, 20, and 25 m depth, and, in Lake Manapouri, they were collected at 25, 50, 100, 200, 300, and 375 m depth. The filtered Cu speciation samples were frozen back in the laboratory for later analysis.

Water for ancillary chemical constituents was sampled into polycarbonate plastic bottles by either hand-dipping or filling directly from the Go-Flo bottles. The cations  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Na}^+$  were determined by inductively coupled

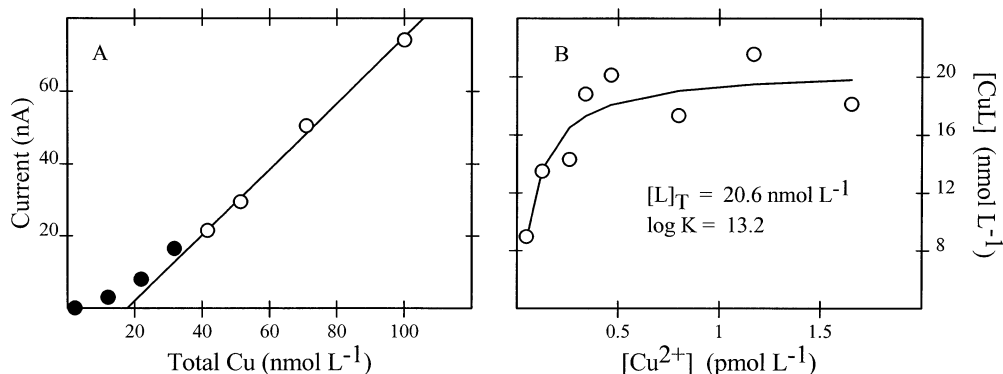


Fig. 1. (A) Titration curve for a lake water sample showing CSV current as a function of the concentration of total (i.e., original + added) Cu. The slope used for calculation of  $[\text{Cu}(\text{HSA})_2]$  was derived from linear regression using the 5 data points shown as open symbols. (B) Nonlinear least-squares fitting of the titration data according to Eq. 11.

plasma optical emission spectrometry, and  $K^+$  was measured by flame atomic absorption spectrometry. The anions  $\text{SO}_4^{2-}$  and  $\text{Cl}^-$  were quantified by ion chromatography. Total alkalinity was determined by computer-controlled potentiometric titration with standard HCl solution ( $0.1024 \text{ mol L}^{-1}$ ), using a Gran function method to locate equivalence points. These measurements were used to compute the ionic strength and inorganic composition of each sample for calculations of  $\alpha_{\text{Cu}}$ .

Total dissolved Cu was determined by two different methods. The first involved subboiling evaporation in Teflon beakers on a hot plate in a clean air bench, which resulted in a preconcentration factor of 10 times. Subsequent determination was by graphite furnace atomic absorption spectrophotometry with a Zeeman Perkin Elmer 4100 ZL spectrometer (Ahlers et al. 1990). The second technique involved ultraviolet (UV) photo-oxidation of a lake water sample and quantification of Cu by CSV using salicylaldoxime with  $\text{Cu}^{2+}$  standard additions (Campos and Van Den Berg 1994).

For the  $\text{Cu}^{2+}$ -ligand titrations, 10.3 ml of lake water sample, 20  $\mu\text{l}$  of  $0.01 \text{ mol L}^{-1}$   $\text{H}_2\text{SA}$  solution, 100  $\mu\text{l}$  of either HEPES-NaOH or HEPPS-NaOH ( $\sim 1 \text{ mol L}^{-1}$ ) buffer, and Cu standard solution (0–120  $\mu\text{l}$ ,  $10 \mu\text{mol L}^{-1}$ ) were equilibrated in Teflon sample cups for 12 h. The pH of each buffer was adjusted by the addition of ultrapure NaOH or HCl solution to match the original lake water within  $\pm 0.2$  pH. These conditions gave a final salicylaldoxime concentration of  $19.8 \mu\text{mol L}^{-1}$ , which corresponded to a side reaction coefficient for the  $\text{Cu}(\text{HSA})_2$  complex (Eq. 5) of  $\log \alpha = 8.5$ .

After equilibration, the samples were analyzed by CSV using either a Princeton Applied Research 303A hanging mercury drop electrode and 264A analyzer or an EcoChemie Autolab PGSTAT-10 and 663 interface connected to a Metrohm 663VA electrode stand. Deposition on a medium-sized Hg drop was at  $-0.050 \text{ V}$  for 1 min, followed by a 15-s equilibrium time and a scan from  $-0.05$  to  $-0.50 \text{ V}$ . With the EG&G system, the current-voltage signal was recorded with a X-Y chart recorder, and the baseline and  $i_p$  (current peak height) were quantified with the aid of French curves. With the Autolab system, the peak height was de-

termined using the standard GPES instrument software system. All electrochemical measurements were conducted inside a clean air bench.

## Results

*Treatment*—Figure 1A shows a typical set of titration results in terms of the measured CSV current as a function of the concentration of  $\text{Cu}^{2+}$  added. Initially, the plot is curved because of competitive binding of  $\text{Cu}^{2+}$  by NOM ligand(s) in the lake water. However, at higher concentrations of  $\text{Cu}^{2+}$ , when no uncomplexed NOM ligand(s) remain, the plot becomes linear. The slope of the linear region was then used to calculate the concentration of SA-bound Cu,  $[\text{Cu}(\text{HSA})_2]$  in each solution. Finally, from a knowledge of the total dissolved Cu concentration in the original lake water,  $[\text{Cu}]_T$ , and that of  $\text{Cu}^{2+}$  added to each solution, the concentration of ligand-bound  $\text{Cu}^{2+}$  was calculated by the difference

$$[\text{CuL}] = [\text{Cu}]_T + [\text{Cu}]_{\text{added}} - [\text{Cu}(\text{HSA})_2] \quad (8)$$

Eq. 3 was recast in the form

$$K'' = \frac{K'}{[\text{H}^+]^n} = \frac{[\text{CuL}]}{[\text{Cu}^{2+}][\text{L}]_T - [\text{CuL}]} \quad (9)$$

where the new conditional formation constant  $K''$  incorporates the pH dependence term and  $[\text{L}']$  has been replaced by the difference between the total ligand concentration  $[\text{L}]_T$  and that bound to Cu. The free  $[\text{Cu}^{2+}]$  at each point was calculated from the measured concentration of  $[\text{Cu}(\text{HSA})_2]$ , and the corresponding value of the side reaction coefficient for formation of the  $\text{Cu}(\text{HSA})_2$  complex was calculated using Eq. 5.

After this, only two unknowns remained in Eq. 9:  $K''$  and  $[\text{L}]_T$ . Traditionally, these are estimated by fitting the titration data to a Langmuir model (Ruzic 1982; Van Den Berg 1982). This involves rearranging Eq. 9 to the linearized form

$$\frac{[\text{Cu}^{2+}]}{[\text{CuL}]} = \frac{1}{K''[\text{L}]_T} + \frac{[\text{Cu}^{2+}]}{[\text{L}]_T} \quad (10)$$

from which a plot of the left-hand side against  $[\text{Cu}^{2+}]$  allows

the determination of  $K''$  and  $[L]_T$  from the slope and intercept. However, this method has occasional problems when a negative intercept is obtained, making it impossible to calculate  $K''$ . Therefore, we adopted the method of Gerringa et al. (1995), which involves rearranging Eq. 10 to the form

$$\frac{[\text{Cu}^{2+}]}{[\text{CuL}]} = \frac{1}{K''[L]_T} + \frac{[\text{Cu}^{2+}]}{[L]_T} \quad (11)$$

Values of  $K''$  and  $[L]_T$  were then obtained by nonlinear least-squares fitting of the right-hand side of Eq. 11 to the titration data (Fig. 1B). In most cases, both methods gave very similar values for  $[L]_T$  but slightly different values of  $\log K''$ .

This data treatment relies on the assumption that  $\text{Cu}^{2+}$  binds to a single ligand. Multiple-ligand versions of Eq. 11 can also be used. Models with more than one ligand will generally always give a better least-squares fit to the data, but the validity of such models needs to be treated with caution. We found that only in some cases was the fit to the data substantially improved by including a second ligand and that the apparent properties thus calculated were usually not reasonable—that is, the  $\log K''$  for the second ligand was extremely large ( $\log K'' > 25$ ). The success of the one-ligand model for our data probably resulted from the fact that our concentration of  $\text{H}_2\text{SA}$  was relatively high, thus favoring the observation of strong Cu-NOM complexes.

In some cases, we found that the data fitting converged to an apparent ligand concentration close to or below that of the total dissolved  $[\text{Cu}]$  in the original sample. In such cases, the titration curve (equivalent to Fig. 1A) was very close to linear. Although we have confidence that such results clearly indicate little or no ligand binding in such a sample, we do not believe that it is possible to place much reliability on the derived  $[L]_T$  and  $\log K''$  values. These situations will be noted in the discussion below.

*Measurement of  $K_1$  and  $K_2$  for  $\text{H}_2\text{SA}$* —Eq. 5 shows that the dissociation constants  $K_1$  and  $K_2$  for  $\text{H}_2\text{SA}$  are required to calculate the side reaction coefficient  $\alpha_{\text{HSA}}$ . Because there was some variation in the literature values, we chose to measure these constants by potentiometric titration of a solution that contained 4 mmol  $\text{L}^{-1}$   $\text{H}_2\text{SA}$  with standard KOH.  $K_1$  and  $K_2$  were calculated from the titration data using a standard nonlinear least squares method (Braibanti et al. 1987). The measurements were made in a series of KCl solutions of increasing ionic strength  $I$ , so that the results could be extrapolated to  $I = 0$  for application to lake waters. Extrapolated results of  $pK_1 = 9.20 \pm 0.07$  and  $pK_2 = 12.3 \pm 0.4$  were obtained (Fig. 2). Details of these measurements will be presented elsewhere.

*Measurement of  $\beta$  for  $\text{Cu}(\text{HSA})_2$* —Use of the side reaction coefficient for the formation of the  $\text{Cu}(\text{HSA})_2$  complex in Eq. 5 requires a knowledge of the formation constant  $\beta$ . We measured this by competitive complexing of  $\text{Cu}^{2+}$  with both  $\text{H}_2\text{SA}$  and ethylene diaminetetraacetic acid using methods described elsewhere for  $\text{Fe}^{3+}$ -HSA complexing (Rue and Bruland 1995) in distilled water medium. Our result, averaged over four experiments, was  $\log \beta = 20.0 \pm 0.3$ . This compares quite well with the value of 15.8 (relative to  $\text{Cu}'$  instead of free  $\text{Cu}^{2+}$ ) reported elsewhere for seawater of sa-

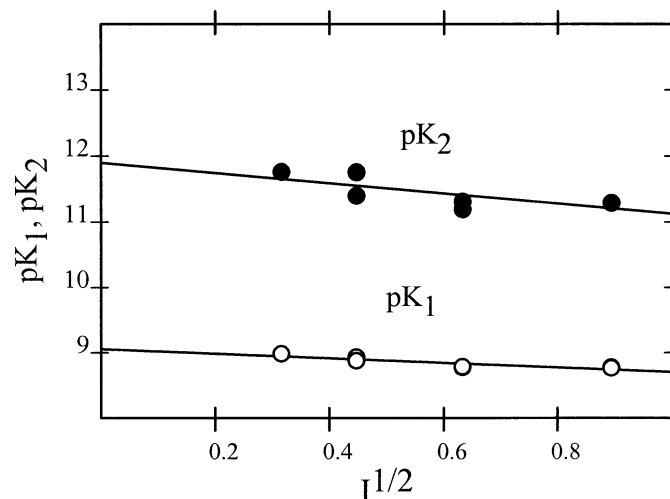


Fig. 2. Measured values of the first ( $pK_1$ ) and second ( $pK_2$ ) proton dissociation constants for salicylaldehyde ( $\text{H}_2\text{SA}$ ) in KCl solutions of different ionic strength  $I$  as a function of  $I^{1/2}$ .

linity  $S = 1$ , pH 8 (Campos and Van Den Berg 1994), after we corrected for the different basis of each conditional constant as follows. We calculated  $\alpha_{\text{Cu}} = 200$  and  $\alpha_{\text{HSA}} = 10$  for pH 8 and then converted our value to conditions of  $\text{Cu}'$  and  $\text{HSA}'$ , gaining a result of  $\log \beta = 20 - \log(200) - 2 \log(10) = 15.7$ .

## Discussion

Figure 3 shows the results obtained for a water sample collected from 5 m depth in Lake Hayes having an in situ pH of 8.1, on which  $\text{Cu}^{2+}$  binding was determined at five different adjusted pH values in the range 6.3–7.9. The pH adjustment was made using noncomplexing HEPES and HEPPS buffers. The conditional  $\text{Cu}^{2+}$ -binding constant  $\log K''$  (Fig. 3A) was observed to increase with pH, with a regression line slope of  $2.17 \pm 0.28$  (standard error of fitting) corresponding to  $n$  in Eqs. 3 and 6. This is very close to the theoretical value  $n = 2$  expected for a ligand that contains independent  $\text{Cu}^{2+}$ -binding functional groups that all have a  $pK_a$  value greater than the highest pH used (pH 7.9). This high  $pK$  value and the preference of  $\text{Cu}^{2+}$  for hard, O-containing ligand donors suggests either phenolic-OH or amino-NH groups as possibilities. In contrast, carboxylic acid groups, although common in NOM, would be completely deprotonated above pH 4–5 and would thus not contribute to any pH dependence of  $\log K''$  in the pH region studied. However, this consideration does not preclude COOH groups being part of a multidentate functional group binding  $\text{Cu}^{2+}$ —for example, a functional group based on the structure of salicylic acid.

Figure 3B shows that the apparent total ligand concentration  $[L]_T$  is not constant as a function of pH, as would be expected if a single class of ligands were being observed.  $[L]_T$  remains reasonably constant in the range  $6.3 < \text{pH} < 7$  but then decreases to slightly  $>75\%$  of the previous values. However, it is probably simplistic to expect there to be a single class of  $\text{Cu}^{2+}$ -binding ligands, because the technique

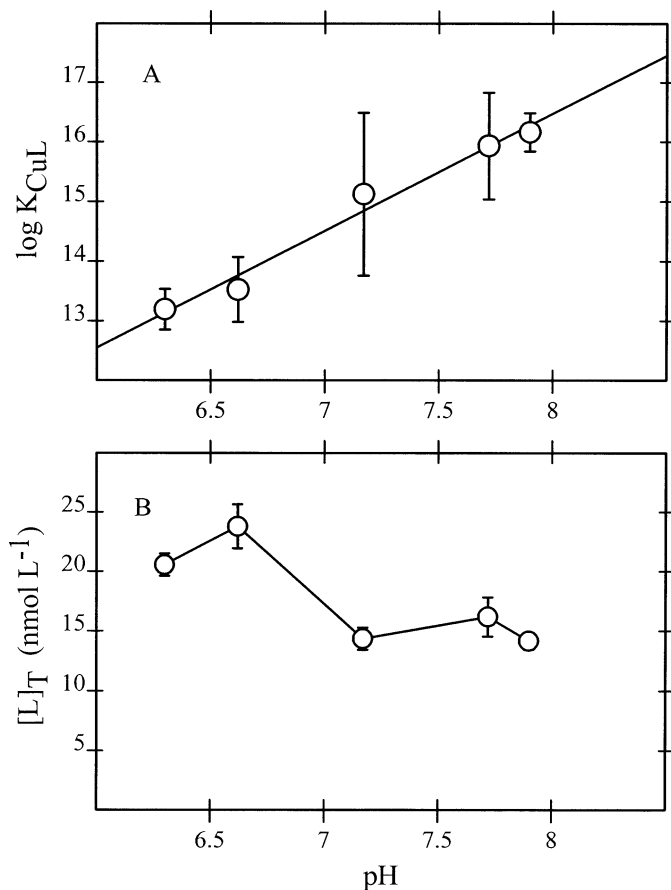


Fig. 3. (A) Conditional CuL formation constant  $\log K''$  and (B) apparent total ligand concentration  $[L]_T$  in a Lake Hayes water sample (5 m depth) measured at five different adjusted pH values. This sample had an in situ pH of 8.1.

we used for determination of  $\text{Cu}^{2+}$  binding has a finite “detection window” (Van Den Berg et al. 1990). Equation 5 indicates that as pH is increased (in the region  $\text{pH} < \text{p}K_1 = 9.2$ ), the side reaction coefficient for  $\text{Cu}(\text{HSA})_2$  increases with pH to the extent of  $2 \log \alpha_{\text{Cu}(\text{HSA})_2}$  units for each unit increase in pH. This means that the detection window is shifting to a larger  $\log K''$  value (by 2 log units per unit pH change). Thus, some classes of ligands may shift out of the detection window at low  $K''$ , and others may shift in at high  $\log K''$ , as the pH is increased. The decrease in apparent  $[L]_T$  observed in Fig. 3B may therefore be evidence of such shifts. Nonetheless, it is clear that the behavior observed in Fig. 3 is not far from that expected for a relatively uniform assemblage of  $\text{Cu}^{2+}$ -binding ligands having weak acid functional groups ( $\text{p}K > 9$ ).

The second method we used to evaluate the effect of pH on  $\text{Cu}^{2+}$  binding was to take advantage of natural variations in pH that occur seasonally and with changes in depth in many lakes. Thus, we collected surface samples on 13 occasions from both lakes during the period April 1997 through March 1999. In addition, we collected samples over the depth range of both lakes on five occasions (January, October, and December 1998 and February and March 1999). For Lake Hayes, the pH of these samples ranged 6.9–

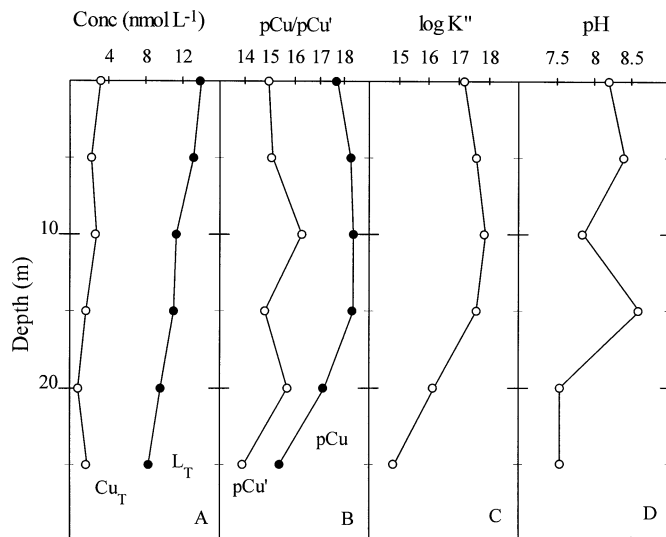


Fig. 4. Copper and copper-binding ligand results for Lake Hayes, October 1998 (austral spring). (A) Concentrations of total dissolved copper  $\text{Cu}_T$  and Cu-binding ligand  $L_T$ . (B) Calculated speciation of free  $\text{Cu}^{2+}$  and  $\text{Cu}'$  (as  $\text{pCu} = -\log[\text{Cu}^{2+}]$  and  $\text{pCu}' = -\log[\text{Cu}']$ , respectively). (C) Conditional Cu-L binding constant  $\log K''$ . (D) In situ water pH. All results shown as vertical depth profiles.

9.1, and that for Manapouri ranged 5.9–9.0. The complete data set for these samples is presented in Web Appendix 1 at [http://www.aslo.org/lo/toc/vol\\_49/issue\\_1/0020a1.pdf](http://www.aslo.org/lo/toc/vol_49/issue_1/0020a1.pdf). Here we present only a selection of the results that are typical of summer and nonsummer conditions.

Figure 4 shows the vertical parameter profiles in Lake Hayes for October 1998 (austral spring): concentrations of both total dissolved copper  $\text{Cu}_T$  and Cu-binding ligand  $L_T$ , the calculated speciation of free  $\text{Cu}^{2+}$  and  $\text{Cu}'$  (as  $\text{pCu} = -\log[\text{Cu}^{2+}]$ ;  $\text{pCu}' = -\log[\text{Cu}']$ , respectively), the conditional Cu-L binding constant  $\log K''$ , and the in situ water pH. Figure 5 shows the corresponding results for March 1999, late austral summer. In October, a slight decrease in pH with depth was observed, which is more enhanced in the late summer results, with almost all of the pH decrease occurring above the hypolimnion. This trend is consistent with the acidification of hypolimnion waters by respiration-derived  $\text{CO}_2$  from sinking biogenic particles. Note also that both pH and the conditional Cu-ligand binding constant,  $\log K''$ , show a closely similar trend with depth on both sampling occasions.

In October, the concentration of Cu-binding ligand exceeded that of dissolved  $[\text{Cu}_T]$  by a factor of  $\sim 4$ – $6$  throughout the water column, which indicates that there is considerable residual Cu-binding capacity. Thus the strong Cu-binding ligand dominates the speciation of  $\text{Cu}^{2+}$ , producing very high pCu values, especially in the oxygenated epilimnion ( $\text{pCu} \approx 17$ – $18$ ). Because the pH of these waters is at least 7.5, side reactions of  $\text{Cu}^{2+}$  with inorganic ions  $\text{OH}^-$  and  $\text{CO}_3^{2-}$  are important, and labile  $\text{Cu}'$  concentrations were 2–3 orders of magnitude higher ( $\text{pCu}' \approx 15$ ).

In March 1999 (Fig. 5), the concentrations of Cu-binding ligand were much lower (except in the deepest sample) but

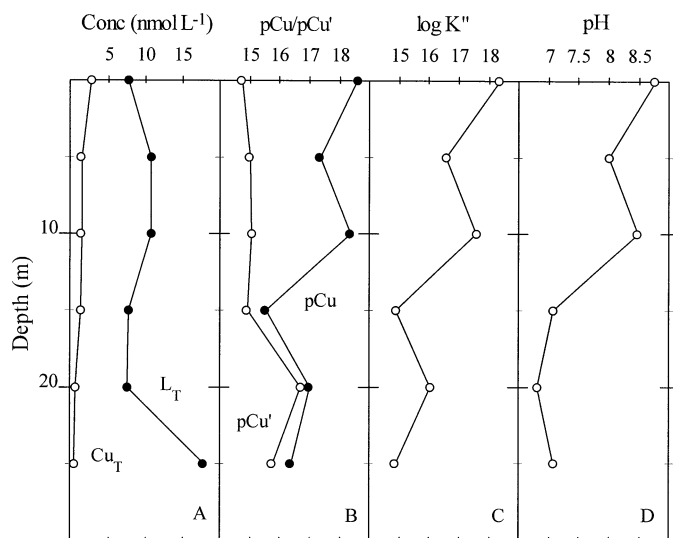


Fig. 5. Copper and copper-binding ligand results for Lake Hayes, March 1999 (late austral summer). Parameters are as described in Fig. 4.

still exceeded  $[Cu_T]$  at all depths. As in October, very low free  $Cu^{2+}$  concentrations were observed (again  $pCu \approx 17$ – $18$ ) in the epilimnion, but, in the deeper waters, higher free  $Cu^{2+}$  concentrations were observed ( $pCu \approx 16$ ), mainly because of the lower  $\log K''$  values at these depths. Because in the deeper water the pH was lower than in October, the inorganic side reactions of  $Cu^{2+}$  with  $OH^-$  and  $CO_3^{2-}$  have been reduced in importance and very little difference between  $pCu$  and  $pCu'$  is observed.

Vertical parameter profiles for December 1998 (Web Appendix 1) showed very similar trends to those presented for October (Fig. 4), whereas those for February (Web Appendix 1) were very similar to those in March (Fig. 5). To summarize, Lake Hayes contains a strong Cu-binding ligand whose concentration exceeds that of total dissolved Cu throughout most of the year, with a conditional  $\log K''$  value that decreases with depth along with pH.

Figures 6 and 7 show the corresponding speciation parameters for Lake Manapouri in October 1998 and March 1999. In October (Fig. 6), concentrations of Cu-binding ligand are generally similar to those in Lake Hayes at the same time (Fig. 4), but, although  $[Cu_T]$  values in Manapouri were significantly higher,  $[L_T]$  remained in excess throughout the water column. Once again, a profile of decreasing pH with depth was seen, in parallel with that of  $\log K''$ . Because the pH of Lake Manapouri water is lower than that of Hayes, inorganic side reactions of  $Cu^{2+}$  are not as important, so that  $pCu$  and  $pCu'$  show only a small difference.

Results in December 1998 for Manapouri (Web Appendix 1) were similar to those in October, but, in January and February 1999 (Web Appendix 1), we observed a significant decrease in  $[L_T]$  in the mixed layer. Indeed, in many cases, the apparent  $[L_T]$  derived from the fitting fell below  $[Cu_T]$ . As we already mentioned, we cannot place much quantitative significance on the derived values of  $[L_T]$ , but we can be reasonably certain that, at these times, there is essentially no control of  $pCu$  by complexing with strong ligands. As is

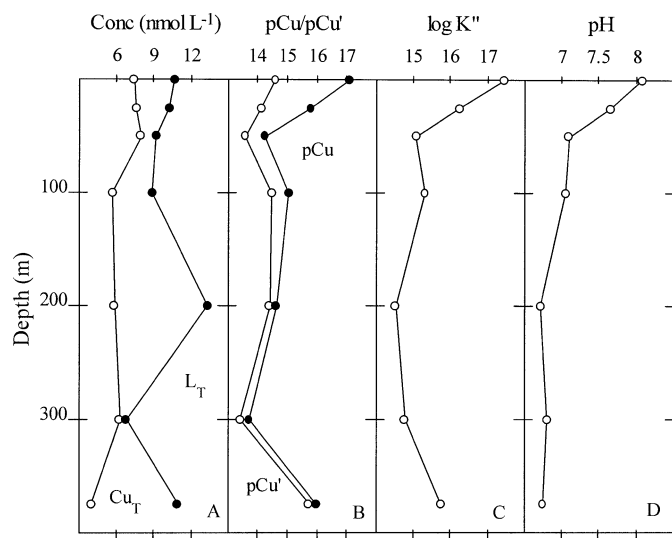


Fig. 6. Copper and copper-binding ligand results for Lake Manapouri, October 1998 (austral spring). Parameters are as described in Fig. 4.

discussed below, this trend was also evident in the temporal variation in surface water concentrations. By March 1999 (Web Appendix 1),  $[L_T]$  appeared to have recovered to levels that once again exceeded  $[Cu_T]$ .

Figure 8 shows the measured  $[Cu_T]$  and  $[L_T]$  in surface waters as a function of sampling date for our complete study (on some sampling dates, we collected only surface samples). These results show that, during two successive summer periods, the Cu-binding ligand seemed to disappear temporarily from the water in Lake Manapouri. Lake Hayes showed a similar, but more pronounced, trend. Several possibilities exist for this interesting result. During late summer, the ozone hole over Antarctica breaks up, exposing southern New Zealand to enhanced UV-B radiation. In addition, solar

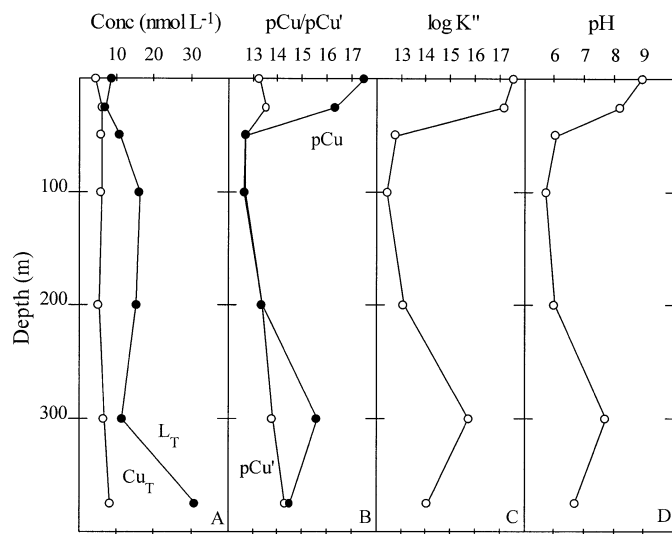


Fig. 7. Copper and copper-binding ligand results for Lake Manapouri, March 1999 (late austral summer). Parameters are as described in Fig. 4.

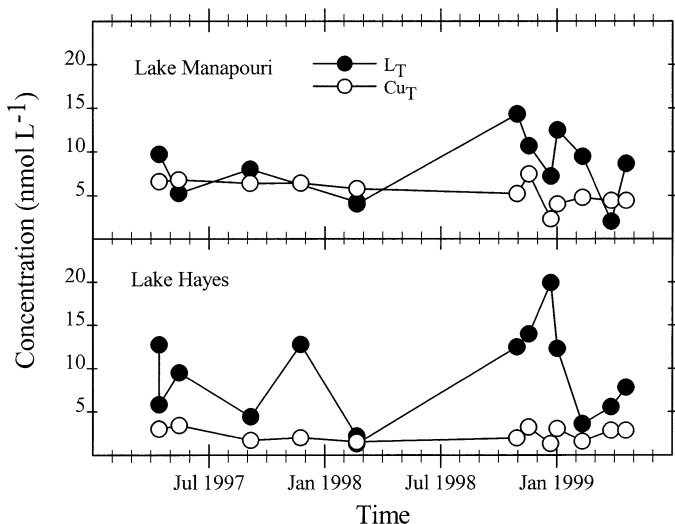


Fig. 8. Comparison of total dissolved copper ( $\text{Cu}_T$ ) and Cu-binding ligand ( $L_T$ ) in surface waters of Lakes Manapouri and Hayes as a function of sampling date.

radiation is generally much more pronounced during this period because of the solar elevation and enhanced daylight hours. Thus, UV irradiation may be responsible for a temporary destruction of NOM ligands. On the other hand, there are pronounced changes in the phytoplankton ecology of these lakes throughout summer and perhaps also of bacterial populations. Thus, the source of NOM ligands may be seasonally dependent. Clearly, further work is required to establish the cause of these changes in Cu-binding ligands.

It is clear that when a strong Cu-binding ligand is present in these lake waters, the free  $[\text{Cu}^{2+}]$  is very low (generally  $p\text{Cu} > 13$ ), making it unlikely that  $\text{Cu}^{2+}$  will induce toxic effects in phytoplankton (Brand et al. 1986; Sunda and Huntsman 1995). However, during the summer periods just mentioned, it is clear that the potential for  $\text{Cu}^{2+}$  toxicity cannot be ignored. Because we used a relatively high concentration of competing ligand  $\text{H}_2\text{SA}$  in our study, we were not able to observe the ligands that form weaker Cu complexes that may be present. Therefore, the  $p\text{Cu}$  values calculated for these cases, which fall mostly in the range  $p\text{Cu} < 11$ , may not be accurate. Thus, more detailed investigations, perhaps using a different CLE ligand, are warranted. However, preliminary measurements we have made more recently using much lower concentrations of  $\text{H}_2\text{SA}$  have not shown significant concentrations of weaker ligands such as we can find in lowland lakes. This may reflect the fact that these two alpine lakes do not receive the same kind of NOM inputs from soil and littoral vegetation that is more characteristic of lowland lake systems.

Finally, we return to the relationship between the conditional Cu-ligand binding constant  $\log K''$  and pH indicated by the experiment with a single Lake Hayes sample, as shown in Fig. 3. Figure 9 shows the combined data for  $\log K''$  for both lakes as a function of pH, taken from the data presented in Figs. 5–8 and Web Appendix 1. This shows that the pH dependence of  $\log K''$  for both lakes are essentially the same, with a calculated slope of  $1.74 \pm 0.11$  ( $r^2$

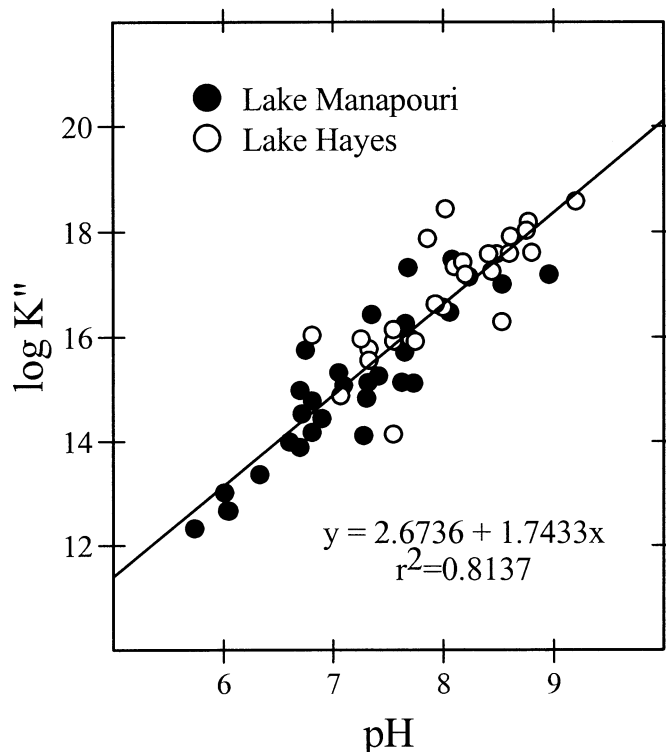


Fig. 9. Conditional CuL formation constant  $\log K''$  as a function of in situ pH for both lakes incorporating all of the samples in the study (Web Appendix 1). The least-squares regression line and equation are shown.

$= 0.8137$ ,  $n = 59$ ), which is slightly lower than that found for the sample whose pH was adjusted ( $2.17 \pm 0.28$ ; Fig. 3B). It is premature to make a definite conclusion on the basis of the measured pH dependence of  $\log K''$  for only a single sample. Nonetheless, this comparison suggests the tentative conclusion that the Cu-binding properties of the ligands observed in both of these lakes, at different depths and different times of the year, may be very similar, at least as far as the proton dissociation reactions of the  $\text{Cu}^{2+}$ -binding functional groups of the ligands are concerned. More detailed study is warranted, particularly if CSV ligands can be found whose detection window does not vary significantly within the pH range of interest.

## References

- ACHTERBERG, E., C. VAN DEN BERG, M. BOUSSEMARY, AND W. DAVISON. 1997. Speciation and cycling of trace metals in Esthwaite Water: A productive English lake with seasonal deep-water anoxia. *Geochim. Cosmochim. Acta* **61**: 5233–5253.
- AHLERS, W. W., M. R. REID, J. P. KIM, AND K. A. HUNTER. 1990. Contamination-free sample collection and handling protocols for trace elements in natural freshwaters. *Aust. J. Mar. Freshw. Res.* **41**: 713–720.
- BRAIBANTI, A., G. OSTACOLI, P. PAOLETTI, L. D. PETTIT, AND S. SAMMARTANO. 1987. Potentiometric apparatus and technique for the pH-metric measurement of metal-complex equilibrium constants. *Pure Appl. Chem.* **59**: 1721–1728.
- BRAND, L. E., W. G. SUNDA, AND R. R. L. GUILLARD. 1986. Re-

- duction of marine phytoplankton reproduction rates by copper and cadmium. *J. Exp. Mar. Biol. Ecol.* **96**: 225–250.
- BRULAND, K. W., J. R. DONAT, AND D. A. HUTCHINS. 1991. Interactive influences of bioactive trace metals on biological production in oceanic waters. *Limnol. Oceanogr.* **36**: 1555–1577.
- CAMPOS, M.L.A.M., AND C. VAN DEN BERG. 1994. Determination of copper complexation in sea water by cathodic stripping voltammetry and ligand competition with salicylaldehydeoxime. *Anal. Chim. Acta* **284**: 481–496.
- COALE, K. H., AND K. W. BRULAND. 1988. Copper complexation in the northeast Pacific. *Limnol. Oceanogr.* **33**: 1084–1101.
- DONAT, J. R., K. A. LAO, AND K. W. BRULAND. 1994. Speciation of dissolved copper and nickel in South San Francisco Bay: A multi-method approach. *Anal. Chim. Acta* **284**: 547–571.
- ELLWOOD, M., K. HUNTER, AND J. KIM. 2001. Zinc speciation in Lakes Manapouri and Hayes, New Zealand. *Mar. Freshw. Res.* **52**: 217–222.
- FLEGAL, A. L., G. SMITH, G. A. GILL, S. SANUDO-WILHELMY, AND L. C. ANDERSON. 1991. Dissolved trace element cycles in the San Francisco Bay estuary. *Mar. Chem.* **36**: 329–363.
- GERRINGA, L. J. A., P. M. J. HERMAN, AND T. C. W. POORTVLIET. 1995. Comparison of the linear Van den Berg/Ruzic transformation and a non-linear fit of the Langmuir isotherm applied to Cu speciation data in the estuarine environment. *Mar. Chem.* **48**: 131–142.
- HUNTER, K. A., J. P. KIM, AND M. R. REID. 1999. Factors influencing the inorganic speciation of trace metal cations in fresh waters. *Mar. Freshw. Res.* **50**: 367–372.
- KOZELKA, P. B., AND K. BRULAND. 1998. Chemical speciation of dissolved Cu, Zn, Cd, Pb in Narragansett Bay, Rhode Island. *Mar. Chem.* **60**: 267–282.
- MOFFETT, J. W., AND L. BRAND. 1996. Production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. *Limnol. Oceanogr.* **41**: 388–395.
- , R. G. ZIKA, AND L. E. BRAND. 1990. Distribution and potential sources and sinks of copper chelators in the Sargasso Sea. *Deep-Sea Res.* **37**: 27–36.
- REID, M., J. KIM, AND K. HUNTER. 1999. Trace metal and major ion compositions of Lakes Hayes and Manapouri. *J. R. Soc. N. Z.* **29**: 245–255.
- RUE, E. L., AND K. W. BRULAND. 1995. Complexation of iron(III) by natural organic ligands in the Central North Pacific as determined by a new ligand equilibration/adsorptive cathodic stripping voltammetric method. *Mar. Chem.* **50**: 117–138.
- RUZIC, I. 1982. Theoretical aspects of the direct titration of natural waters and its information yield for trace metal speciation. *Anal. Chim. Acta* **140**: 99–113.
- SUNDA, W. G., AND R. L. GUILLARD. 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *J. Mar. Res.* **34**: 511–529.
- , AND S. A. HUNTSMAN. 1995. Regulation of copper concentration in the oceanic nutricline by phytoplankton uptake and regeneration cycles. *Limnol. Oceanogr.* **40**: 132–137.
- TANFORD, C. 1961. *The physical chemistry of macromolecules*. Wiley-Interscience.
- VAN DEN BERG, C. M. G. 1982. Determination of copper complexation with natural organic ligands in seawater by equilibration with MnO<sub>2</sub>. Part 1: Theory; Part 2: Experimental procedures and application to surface seawater. *Mar. Chem.* **11**: 307–342.
- , M. NIMMO, P. DALY, AND D. TURNER. 1990. Effects of the detection window of the determination of organic copper speciation in estuarine waters. *Anal. Chim. Acta* **232**: 149–159.
- XUE, H., D. KISTLER, AND L. SIGG. 1995. Competition of copper and zinc for strong ligands in a eutrophic lake. *Limnol. Oceanogr.* **40**: 1142–1152.
- , A. OESTREICH, D. KISTLER, AND L. SIGG. 1996. Free cupric ion concentrations and Cu complexation in selected Swiss lakes and rivers. *Aquat. Sci.* **58**: 69–87.
- , AND L. SIGG. 1993. Free cupric ion concentration and Cu(II) speciation in an eutrophic lake. *Limnol. Oceanogr.* **38**: 1200–1213.
- , AND W. SUNDA. 1997. Comparison of [Cu<sup>2+</sup>] measurements in lake water determined by ligand exchange and cathodic stripping voltammetry and by ion-selective electrode. *Environ. Sci. Technol.* **31**: 1902–1909.

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