Fall 1995

Copper Complexation Capacity and Copper Speciation in Freshwater Lakes (Lake Western Branch and Lake Prince)

Rachide Ibrahimo Abdul Sultana
Old Dominion University

COPPER COMPLEXATION CAPACITY AND COPPER SPECIATION IN FRESHWATER LAKES (LAKE WESTERN BRANCH AND LAKE PRINCE)

by

Rachide Ibrahimo Abdul Sultana

B.S. August 1989, Universidade Eduardo Mondlane of Maputo

A Thesis submitted to the Faculty of

Old Dominion University In Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

CHEMISTRY

OLD DOMINION UNIVERSITY

December, 1995

Approved by:

Dr. John R. Donat (Director)

Dr. Frank E. Scully, Jr.

Dr. Gary C. Schafran

ABSTRACT

COPPER COMPLEXATION CAPACITY AND COPPER SPECIATION IN FRESHWATER LAKES (LAKE WESTERN BRANCH AND LAKE PRINCE)

Rachide Ibrahimo Abdul Sultana

OLD DOMINION UNIVERSITY, 1995

Director: Dr. John R. Donat

This research examines the copper complexation capacity, copper speciation and dissolved copper concentrations in Lake Western Branch (WB) and Lake Prince (LP) both located in Suffolk, Virginia. These lakes are drinking water reservoirs for the cities of Suffolk and Norfolk, and they experience blooms of algae which can deplete them of oxygen and affect treatment of their waters for drinking. Copper sulfate has regularly been added to control the algae, but the effectiveness of these additions is uncertain. For environmental management implications, knowledge of speciation (i.e., the concentrations of the various chemical forms) is important in studying the effects of copper (and other trace metals) on algae. Trace metals in natural waters can be complexed by inorganic and organic ligands, and their chemistry can be affected by phytoplankton in different ways: by surface reactions, metal uptake, and production of exudates (extracellular organic matter) with metal complexing properties. Therefore, the uncertain effects of trace metals on algae result from lack of definitive knowledge of the actual chemical forms (i.e., free ions, inorganic and organic complexes) of copper in these lakes, and their concentrations.

Information on copper speciation will lead to a more complete understanding of the interactions of copper with aquatic biota and its biogeochemical cycling.

A very sensitive and accurate analytical technique capable of distinguishing the forms of copper and their concentration levels in natural waters, ligand competition/ differential pulse cathodic stripping voltammetry (LC/DPCSV), was used in this research. This technique has several advantages over others for determining trace metal concentrations and speciation.

Surface (2 m) lake water samples were collected in different seasons: Fall 1994, Spring 1995, and Summer 1995 at four stations in Lake Western Branch and three stations in Lake Prince. Total dissolved copper ranged from 7.13$\pm$ 0.11 to 65.25$\pm$0.74 nM in Lake Western Branch, and from 5.96$\pm$0.60 to 59.2$\pm$0.29 nM in Lake Prince. LC/DPCSV determinations of the natural copper complexing organic ligands in Lake Western Branch and Lake Prince indicated the presence of two classes of ligands. In Lake Western Branch, the stronger class, L_1, had concentrations (C_{L1}) ranging from 33.33$\pm$0.10 to 83.44$\pm$0.04 nM, with a conditional stability constant (expressed with respect to Cu^{2+}) ranging from (log K'_{CuL1} values) 12.45$\pm$0.37 to 14.71$\pm$0.03. The weaker class, L_2, in Lake Western Branch had concentrations (C_{L2}) ranging from 27.76$\pm$0.25 to 49.72$\pm$0.19 nM, with log K'_{CuL2} values ranging from 7.27$\pm$0.08 to 8.25$\pm$0.30. In Lake Prince, C_{L1} ranged from 26.51$\pm$0.96 to 78.74$\pm$0.66 nM, and log K'_{CuL1} ranged from 12.43$\pm$0.14 to 14.58$\pm$0.04. C_{L2} ranged from 10.81$\pm$0.47 to 16.56$\pm$0.44 nM, and log K'_{CuL2} ranged from 7.98$\pm$0.76 to 8.35$\pm$0.29. The results indicate that over 99 % of the total dissolved copper in the water surface (2 m) of both lakes is organically complexed, and copper speciation is dominated by organic complexes

formed with the stronger ligand class, L_1. Because of the high extent of organic complexation, fractions of inorganic copper are reduced to less than 2 % of the total dissolved copper. Free cupric ion concentrations ranged from $0.35 \pm 0.02 \times 10^{-14}$ M to $0.83 \pm 0.05 \times 10^{-9}$ M in Lake Western Branch, and from $0.113 \pm 0.006 \times 10^{-14}$ to $15.4 \pm 0.22 \times 10^{-13}$ M in Lake Prince.

ACKNOWLEDGEMENTS

I thank Dr. John Donat, my thesis advisor, for his infinite support, technical skills, and his patience and guidance throughout the lengthy process of this work. His tremendous enthusiasm is an important source of motivation for me to undertake this work.

I would like to thank the members of my thesis committee, Dr. F. Scully for assisting me in all my needs, and to Dr. G. Schafran for help and advice on my project.

My appreciation is extended to those who assisted me in this project: to Dr. S. Skrabal, and T. Henry.

To R. Miranda, I really appreciate all patience,love and help during my difficulties.

To my family:

I deeply express my appreciation for

their patience, encouragement and love

all the time.

TABLE OF CONTENTS

page

LIST OF TABLES vi

LIST OF FIGURES vii

CHAPTER

I. INTRODUCTION 1

1.1 Overview 1

1.2 Rationale 4

II. COPPER COMPLEXATION AND SPECIATION IN NATURAL WATERS 5

2.1 Overview 5

2.2 Analytical methods for studying copper speciation in natural waters 10

2.2.1 Ligand competition-differential pulse cathodic stripping voltammetry (LC/DPCSV) 14

III. METHODS AND MATERIALS 21

3.1 Instrumentation 21

3.2 Reagents 22

3.4 Analysis 26

3.4.1 Total dissolved copper determinations 26

3.4.2 Copper speciation determinations 27

3.4.3 Determination of α_{CuSA} 34

IV. RESULTS AND DISCUSSION 38

4.1 Comparison of various competing ligands 39

4.2 Linear titration range 40

4.3 Total dissolved copper 43

4.4 Copper complexing ligands and conditional stability constants 43

V. CONCLUSIONS 55

VI. REFERENCES 57

LIST OF TABLES

TABLE *page*

1. DPCSV determinations of total dissolved copper in standard reference materials 28

2. Temperature, pH, total dissolved copper concentrations (Cu_T), and free Cu^{2+} concentrations in Lakes Western Branch and Prince surface waters (2m depth) in Sept.'94, and March, April, and July '95 . . . 44

3. Copper-complexing ligand concentrations and conditional stability constants, and copper speciation in surface waters of Lake Western Branch - April 1995 47

4. Copper-complexing ligand concentrations and conditional stability constants, and copper speciation in surface waters of Lake Western Branch - July 1995 48

5. Copper-complexing ligand concentrations and conditional stability constants, and copper speciation in surface waters of Lake Prince - April 1995 49

6. Copper-complexing ligand concentrations and conditional stability constants, and copper speciation in surface waters of Lake Prince - July 1995 50

LIST OF FIGURES

FIGURE *page*

1. Factors controlling the concentrations of trace metals in freshwater lakes . . 3

2. A model showing possible interactions of copper in a natural water 9

3. Lake Western Branch station locations 23

4. Lake Prince station locations 24

5. (a) Copper titration curve of sample, WB-X, 2 m depth, July 1995 30
   (b) Linearization of WB-X sample showing the presence of only one ligand 31
   (c) Copper titration curve of sample, WB-X, 2 m depth, April 1995 32
   (d) Linearization of WB-X sample showing curvature indicating that two copper-complexing ligands are present 33

6. Effect of EDTA on the DPCSV copper reduction current (sample: WB-3) 36

7. Effect of EDTA on the DPCSV reduction current (sample: LP-2) 37

8. LC/DPCSV copper reduction current in Lake Western Branch as a function of copper concentration 41

9. LC/DPCSV copper reduction current in Lake Prince as a function of copper concentration 42

10. Total dissolved copper in Lakes Prince and Western Branch 45

11. Concentrations of ligand L_1 in Lakes Prince and Western Branch 51

12. Free Cu^{2+} concentrations in Lakes Prince and Western Branch 53

I. INTRODUCTION

1.1 Overview

Concentrations of dissolved copper in natural waters range from 10^{-9} (nanomolar, nM) to 10^{-12} (picomolar, pM), and are controlled by a range of chemical processes (Salbu and Steinnes, 1995). For example, adsorption/ desorption, complexation, or oxidation/ reduction reactions are recognized to be significant in the geochemical cycles (transport, precipitation) and biological cycles (bioavailability, bioconcentration, bioaccumulation, and toxicity) of elements in natural water (Morrison, 1989; Florence, 1989; Sunda, 1994). Dissolved copper can exist in various forms (species): as free, solvated forms ($Cu(H_2O)_6^{2+}$), as inorganic complexes with carbonate, hydroxide, sulfate, and chloride, and as complexes with various organic ligands (e. g. phytoplankton metabolites, amino acids, humic and fulvic acids, etc.). Copper may also exist in colloidal forms which can contribute to its fate and transport (Turner et al., 1981; Byrne et al., 1988; Morrison, 1989). The interactions of copper with aquatic biota are determined by the distribution of the total copper concentration amongst its various forms (speciation). Knowledge of the speciation of the elements in natural waters has become increasingly important because the biogeochemical cycling of an element is dependent on its physico-chemical form (Morrison, 1989); for example, metal toxicity to phytoplankton is controlled by its free metal ion activity rather than the total concentration (Sunda and Guillard, 1976; Anderson and Morel, 1982). Interactions between copper ions and dissolved organic ligands in natural waters can strongly influence copper speciation (Coale and Bruland, 1988; Moffett et al., 1990; Donat and Bruland, 1992; Donat, 1994)

and can decrease the toxicity and bioavailability of copper to biota and increase or decrease the adsorption of copper onto particles.

More than 75 % of copper entering most freshwater lakes comes from the lake drainage area, and several processes regulate concentrations of all species present (Figure 1) (Salomons and Baccini, 1986). In these aquatic reservoirs, information on trace metal speciation is limited. Knowledge of the speciation of copper in freshwater lakes, especially the extent to which copper is complexed by dissolved organic ligands, is important to understanding the toxicity and biological cycling of copper (and other metals) in these systems. Assessments of impacts of high metal concentrations on aquatic biota, including intentional additions of toxic metals (e. g., addition of copper sulfate to control algal growth in drinking water reservoirs), based upon total metal concentrations, are misleading because not all forms of these metals are toxic, or are not toxic to the same extent. In Lake Western Branch and Lake Prince knowledge of copper speciation will contribute not only to a better understanding of trace metal speciation and metal biogeochemical cycling in freshwater lakes, but will also be important to management and policy recommendations concerning the water quality and recreational uses of these suburban lakes.

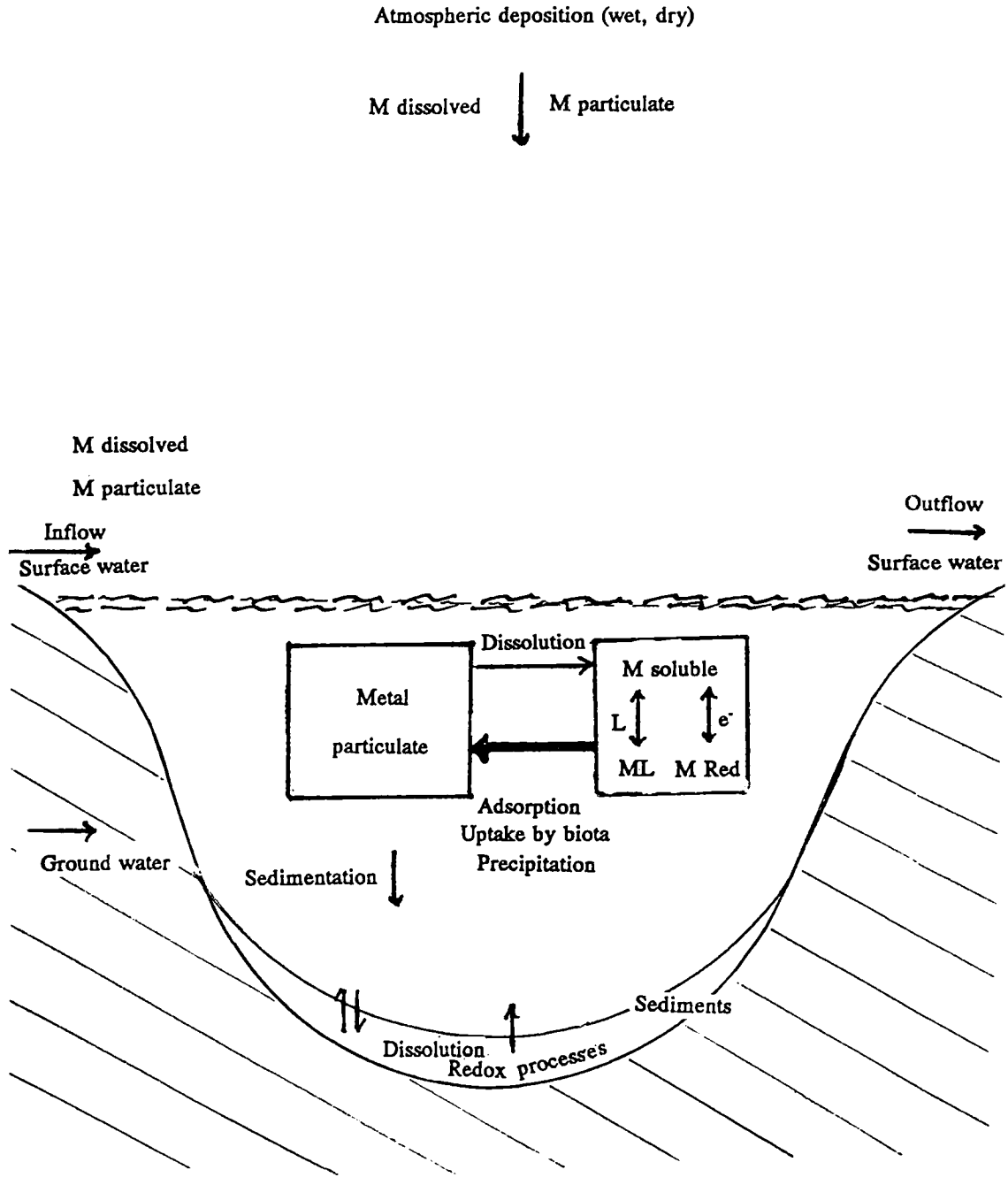


Figure 1. Factors regulating the concentrations of trace metals in a freshwater lake (Sigg, 1985).

1.2 Rationale

Lake Western Branch (≈ 38.64 Km^2) and Lake Prince (≈ 16.4 Km^2), both located in the South Hampton Roads region of Virginia, were chosen for this study because nothing is known about concentrations of total dissolved copper, and the free cupric ions, the concentrations and strengths of the copper-ligands present, the extent to which copper is organically complexed, nor the importance of copper in these, or any other, Virginia lakes. These lakes, which are sources of drinking water and sites of recreation for the cities of Suffolk and Norfolk, are treated with copper sulfate in an effort to control algal growth. However, copper sulfate treatment seems to be only partially effective. These blooms make them aesthetically displeasing (algal mats and odor), deplete them of oxygen causing fish mortality, and can affect their treatment as drinking water sources.

From an environmental policy point of view, copper speciation studies in these lakes approaches an issue that has not yet been addressed by the appropriate water authorities before, and should be included as part of their water quality programs because Lake Western Branch and Lake Prince are faced with pressure from suburban development of the land surrounding them, and from agricultural and residential run off.

II. COPPER COMPLEXATION AND SPECIATION IN NATURAL WATERS

2.1 Overview

Trace metals in natural waters occur in different physical phases: dissolved, particulate, or colloidal, each with different biological and geochemical behaviors. These phases are differentiated using 0.45 μm (or 0.22 μm) pore size filters. Within the dissolved phase, trace metals can exist as different chemical forms (species): as the free, hydrated ion ($M(H_2O)_n^{2+}$), complexes with inorganic ligands (MX_i), and complexes with organic ligands (ML_i). As with the different physical phases, these dissolved species can have different biological and geochemical reactivities and effects.

The concentrations of the free metal aquo ions regulate the availability and toxicity of metals to biota in natural waters. For example, Cu^{2+} ions at concentrations ranging from 10^{-12} - 10^{-10} M and free Cd^{2+} ions at concentrations of 10^{-10} M are toxic to some marine phytoplankton, and Cd^{2+} ions are toxic to grass shrimp (Sunda and Guillard, 1976; Sunda et al.,1978; Brand et al., 1986). The availabilities of the nutrient metals Fe, Mn, and Zn to phytoplankton (Brand et al., 1983) are also regulated by the concentrations of their respective free metal ions rather than the total concentration of each metal. Many aquatic organisms show toxic responses even to relatively low free Cu^{2+} concentrations (Sunda et al., 1990). Stoecker et al. (1986) showed the limits of copper toxicity to marine planktonic ciliates to be 10^{-10} to 10^{-13} M. In freshwater, copper was found to be toxic in the range 10^{-10} to 10^{-12} M to the algae *Scendesmus quadricauda.* Zevenhuisen et al. (1979) observed that the freshwater bacterium *Klebsiella aerogenes*

exhibits toxic responses to copper at concentrations of 10^{-6} to 10^{-10} M.

Brand et al. (1986) reported that while prokaryotic marine cyanobacteria are sensitive to copper at concentrations greater than 10^{-10} M, diatoms are least sensitive and other groups (coccolithophorids, dinoflagellates) show an intermediate response. Interestingly, cyanobacteria are the main nuisance-bloom-causing algal group in Lakes Western Branch and Prince (Schafran and Scully, 1993), and seasonal variations in copper speciation may influence the biological productivity of different phytoplankton species.

Metal toxicity can be reduced by complexation with inorganic and organic ligands. For example, Morrison (1989) reported that for some crustaceans such as *Daphnia magna*, Cu^{2+} and $CuOH^{2+}$ are toxic, while carbonato-copper complexes are nontoxic. Organic complexation has been shown to decrease toxicity of copper to marine phytoplankton (Sunda et al., 1990; Sunda, 1990). In addition, organic complexation can influence exposure of aquatic biota to copper by increasing or decreasing adsorption of trace metals onto particles. Sunda et al. (1990) described the increasing survival of copepod larvae in estuarine waters having high free cupric ion concentrations due to additions of complexing agents such as EDTA and NTA.

Inorganic complexes can be formed with ligands such as hydroxide, carbonate, and sulfate. Turner et al. (1981), and Byrne et al. (1988) modelled the inorganic speciation of metals in natural waters and described extensively the inorganic complexes of metals based on thermodynamic and environment variability in aquatic systems. Over 90 % of inorganic copper in freshwater lakes is believed to be present as $CuCO_3$ (Florence, 1989), and the other portions associated with colloidal particles because of the high pH.

Organic complexation of metals has been studied more extensively in oceans than in freshwaters (Xue and Sigg, 1993). In open oceans, coastal, and estuarine waters organic complexation dominates the speciation of dissolved copper and, therefore, controls free cupric ion concentrations (van den Berg, 1984; Donat et al., 1986; Moffett and Zika, 1987; Sunda and Hanson, 1987; Coale and Bruland, 1988; Donat, 1994; Donat and Bruland, 1995). These studies generally show that 80 % (usually $\geq$ 99%) of total dissolved copper in surface seawater is complexed by very strong natural organic ligands (log K'_{CuL}: 10 - 14) existing at very low (10^{-9}, nM) concentrations. In seawater, this level of organic complexation buffers cupric ion concentrations at about 10^{-13} M.

In freshwater lakes, dissolved copper concentrations range from approximately 2 - 150 nM, and recent studies have shown that 99.7 % of dissolved copper (and zinc) exist as organic complexes (Borg, 1995). In a eutrophic Swiss lake, higher concentrations of strong ligands (40 - 556 nM) higher than those in seawater were observed having conditional stability constants in the same range as those of estuaries (log K_1 11.8 to 14.9) (Xue and Sigg, 1993). The concentrations and strengths of these organic ligands reduced the free Cu^{2+} concentration in this Swiss lake to 10^{-14} to 10^{-16} M.

The identities of the ligands forming trace metal complexes in freshwaters are not known because they have never been isolated and characterized. In seawater they exist at very low concentrations ($\approx$1 - 100 nM) with high stability constants ($\approx 10^{10}$ - 10^{13}) for copper complexation (see review by Donat and Bruland, 1995). These ligands may include simple organic molecules (e.g. amino acids, oxalic acids) and macromolecular compounds (e.g. humic, fulvic, and tannic acids) (Morel and Hering, 1993). Other ligands present in freshwater and seawater are also excreted by microorganisms or algae that possibly change the speciation of copper and other metals. For example,

siderophores are strong ligands for copper (II) (Sigg and Xue, 1994) and very specific for iron (II) (McKnight and Morel, 1979; Morel and Hering, 1993). Synthetic organic ligands as NTA (nitrilotriacetate) and EDTA (ethylenediaminetetraacetate) may also exist in natural waters because of anthropogenic activities.

Figure 2 represents a conceptual model of free cupric ion concentrations in a natural water. In a typical freshwater, macromolecular organic compounds are frequently the dominant ligands and take part in complexation and acid base reactions (Buffle, 1988).

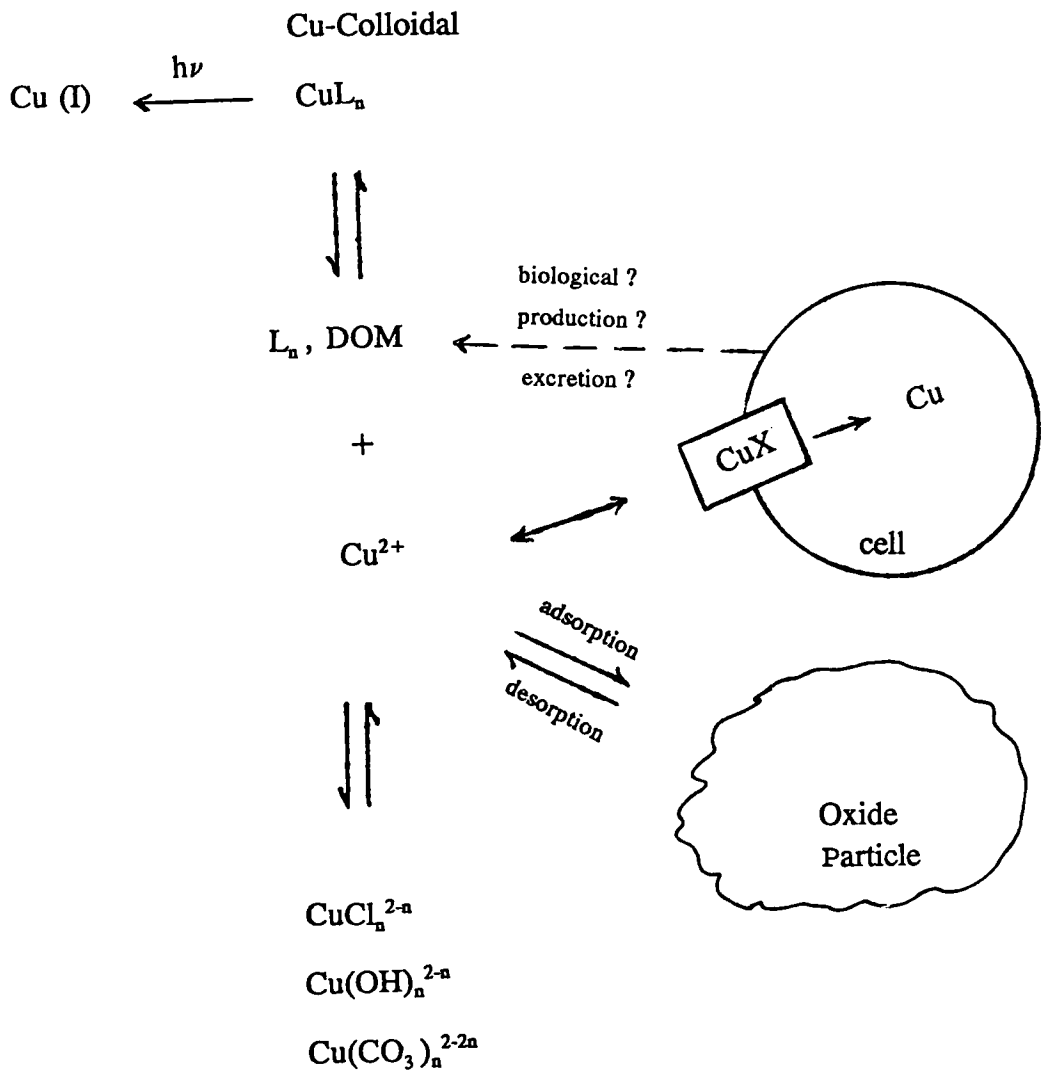


Figure 2. A model showing possible interactions of dissolved copper in a natural water.

2.2 Analytical methods for studying copper speciation in natural waters

Several analytical methods have been used to measure copper complexation and speciation in natural waters. Some of them are suitable but others lack sensitivity for these studies. They can be direct or indirect methods and each has advantages, disadvantages, and/or theoretical or practical limitations. Direct methods isolate or detect one of the metal fractions originally present in a natural water sample. Indirect methods isolate or detect a metal fraction not originally present in the sample, but created for the speciation determinations. Indirect methods require establishment of a competitive equilibrium between the metal, the natural organic ligands, and a competing organic ligand, particle or resin surface added to aliquots of the sample (Donat and Bruland, 1995).

Anodic stripping voltammetry (ASV) involves titration of a water sample with a metal, and then determination of concentration of inorganic metal in equilibrium with metal organic complexes. ASV has frequently been used for determinations of copper concentrations and speciation in natural waters (Duinker and Kramer, 1977; Donat et al., 1986; Coale and Bruland, 1988; 1990; Donat and Bruland, 1992; Donat et al., 1994; Donat, 1994) because of its relatively high sensitivity and ability to distinguish between labile (i.e., reactive) species (usually inorganic species such as Cu^{2+}, inorganic complexes: e.g., $CuCO_3$, $CuOH^+$, $Cu(OH)_2$, etc., and weak organic complexes: e.g., copper citrate, copper glycinate) and nonlabile, strongly-bound organic complexes; ASV directly detects labile copper (as defined above), but does not detect non-labile, strongly bound (usually organic) copper complexes. However, it lacks the sensitivity to directly determine the very low (10^{-12} - 10^{-14} M) concentrations of inorganic copper existing in

natural waters containing strong copper complexing ligands.

Another technique for copper speciation is fixed-potential amperometry (FPA) which detects the total dissolved inorganic copper. FPA avoids direct reduction of labile organic complexes (Waite and Morel, 1993; Hering et al., 1987); however, it is applicable only in solutions containing high chloride and relatively low ligand concentrations ($\leq$ 100 nM).

Metal ion selective electrodes (ISE) can be used to measure directly the free Cu^{2+} activity in waters where these concentrations are high ($\approx 6.1 \times 10^{-7}$ M), but chloride can interfere (>0.1 M) (Belli and Zirino, 1993). Therefore, their sensitivity and selectivity are insufficient for most natural waters with low free metal concentrations.

In seawater and freshwaters containing lower concentrations of total dissolved copper (≈ 0.25 - 1 nM) biological assays provide direct information of free ion activity (Sunda and Ferguson, 1983; Anderson et al., 1984; Hering et al., 1987). They are based on calibration of Cu^{2+} toxicity to bacteria or phytoplankton. However, bioassay techniques can underestimate copper complexation at such low levels.

Solid phase extraction (SPE) involves sorption of hydrophobic copper complexes onto C_{18} Sep-Pak cartridges. These are prepacked columns filled with a resin containing octadecylsilyl groups bonded to a silica gel support. This method was developed by Mills and Quinn (1981) and Hanson and Quinn (1983), who observed that up to 60 % of the dissolved copper in Narragansett Bay and coastal Atlantic waters could be isolated. These workers claimed that the fraction of organically-bound copper isolated by Sep-Pak cartridges was "the most geochemically-significant" fraction, even though it probably was not the total amount of organically-bound copper in these waters. Donat et al. (1986) critically evaluated the Sep-Pak method, and found that it separates only the fractions of

organically complexed copper that are sufficiently hydrophobic to be adsorbed by the C_{18} resin. This fraction represents only $\approx 10\%$ of organically-complexed copper in oceanic sample waters, but Sep-Paks recover ≈ 60 % of organically-complexed copper in deeper oceanic waters. Later, Sunda and Hanson (1987) improved this method by ligand competition with EDTA, in which the concentration of Cu^{2+} is determined after adsorption of Cu^{2+} onto Sep-Pak C_{18} cartridges. An excess of EDTA (which competes with natural ligands for copper complexation) is added to produce a cupric ion buffer to calibrate interactions between the free Cu^{2+} concentrations and the Sep-Pak cartridges. This C_{18} Sep-Pak technique with internal Cu^{2+} ion calibration can be used for measuring copper complexation and free cupric ion concentration in both seawater and freshwater, but it is a complicated and time consuming method.

Exchange with solid MnO_2 is another indirect method which involves addition of finely-divided solid MnO_2, which adsorbs only inorganic copper, to the sample to equilibrate with copper and the natural ligands (van den Berg, 1982; van den Berg and Dharmvanij, 1984). The sample is filtered to remove the solid MnO_2, and the copper concentration remaining in the filtrate (a measure of non-adsorbable, organically complexed copper) is determined by a suitable method (e.g. atomic absorption, ASV, etc.). The free Cu^{2+} ion concentrations present naturally in the sample are calculated from equilibrium considerations.

Exchange with ion-exchange resins is based on the rate of retention of metal and labile forms by a resin (Mackey, 1982; Sunda, 1984; Donat et al., 1994); organically-complexed metals are not adsorbed.

Ligand competition (LC) methods are based on competition for copper between the organic ligands present naturally in the sample and well-characterized organic ligands

added to the sample, and the subsequent specific determination of concentrations of either free copper or of the complexes formed between copper and the added ligands. The original copper speciation is then determined by equilibrium calculations. Ligand competition methods have been coupled with many separation and detection methods including (1) ligand-exchange (e.g., catechol, 8-hydroxyquinoline, tropolone, salicylaldoxime, benzoylacetone) and adsorptive cathodic stripping voltammetry of copper added-ligand complexes in seawater (van den Berg, 1984; Buckley and van den Berg, 1986; Donat and Bruland, 1992; Donat and van den Berg, 1992; van den Berg and Donat, 1992), in estuarine waters (van den Berg et al., 1990; Donat et al., 1994; Donat, 1994), and in lake water (Xue and Sigg, 1993); (2) ligand (EDTA) competition and adsorption of free Cu^{2+} onto C_{18} Sep-Pak cartridges mentioned above (Sunda and Hanson, 1987), (3) ligand (EDTA) competition with chemiluminescence detection of free Cu^{2+} concentrations in seawater (Sunda and Huntsman, 1991), and (4) ligand (acetylacetone) competition, solvent/ solvent partitioning (Moffett and Zika,1987).

2.2.1 Ligand competition-differential pulse cathodic stripping voltammetry (LC/DPCSV)

In this research, copper complexation and speciation were determined using ligand competition/ differential pulse cathodic stripping voltammetry (LC/DPCSV). It has a low detection limit (10^{-10} - 10^{-12} M), and good precision and accuracy for measuring trace metals. LC/ DPCSV does not require a separate preconcentration step, requires only a small amount of sample (5-20 mL), and requires relatively inexpensive instrumentation. This method utilizes an equilibration competition for copper between the natural copper ligands in the sample and a well characterized organic ligand (AL: e.g., salicylaldoxime (SA), Campos and van den Berg, 1994; catechol, van den Berg, 1984; van den Berg and Donat, 1992; 8-hydroxyquinoline (8-HQ), van den Berg et al., 1990; Donat and Bruland, 1992; Donat et al., 1994; Donat, 1994; tropolone, Donat and van den Berg, 1992; or benzoylacetone, Moffett, 1995), added to aliquots of a buffered sample at a known and constant concentration:

CuX_n (copper inorganic complexes)

$$\begin{matrix} X_n \\ + \\ Cu^{2+} + L_n \\ + \\ AL_n \end{matrix} \quad \begin{matrix} \\ \\ CuL_n \text{ (natural organic copper complexes)} \\ \\ \\ CuAL_n \text{ (copper-added ligand complexes)} \end{matrix} \qquad (1)$$

where reactions between Cu^{2+} and X_n and L_n are the equilibria occurring naturally in the sample. X represents important inorganic ligands such as CO_3^{2-}, OH^-, HCO_3^-, SO_4^{2-}, Cl^-. L represents natural organic ligands. The equilibrium between Cu^{2+} and AL is established after addition of the added ligand (AL).

In LC/DPCSV, a series (usually 5 to 10) of 10 mL aliquots of the sample are first spiked with incrementally-increasing concentrations of copper which are allowed to equilibrate with the natural organic ligands for $\approx$8 hours. A known and constant concentration of the added ligand is then added to each of the copper-spiked sample aliquots to establish a competing equilibrium for copper during 12-15 hours. The copper added-ligand complexes in each aliquot are then adsorbed to the surface of a hanging mercury drop electrode (HMDE) whose potential is set by a potentiostat to support adsorption. After an appropriate adsorption time, the potential on the HMDE is ramped toward more negative values, and current resulting from the reduction of cupric ions in the adsorbed copper-added ligand complexes on the HMDE is then measured. The reduction current thus obtained is proportional to the concentration of copper in the sample that is complexed by the added ligand. The amount of copper complexed by the added ligand is a function of the concentration of added ligand and the conditional stability constant of the copper added-ligand complexes. The peak current resulting from the reduction of copper complexed by the added ligand is measured for each added copper concentration and is plotted vs. the total copper concentration (natural + added) in each aliquot to produce a titration curve. The total concentration of copper-complexing ligand, C_L, in the sample, and the conditional stability constant of its copper complexes, $K'_{CuL,Cu2+}$, are calculated from linearization data using the following equation (van den Berg and Kramer, 1979; Ruzic, 1982; van den Berg, 1984):

$$[Cu^{2+}]/[CuL] = [Cu^{2+}]/C_L + 1/(K'_{CuL,Cu2+} \times C_L) \quad (2)$$

which is derived from the mass balance for total ligand:

$$C_L = [L'] + [CuL] \quad (3)$$

and the equilibrium expression for formation of the natural copper-organic complexes:

$$K'_{CuL,Cu2+} = [CuL]/([Cu^{2+}] \times [L']) \quad (4)$$

where C_L is the total concentration of organic copper-complexing ligand ("total copper-complexing capacity");

[L'] is the concentration of organic ligand not bound to copper ("excess copper-complexing capacity");

[CuL] is the concentration of the natural copper-organic complexes;

$[Cu^{2+}]$ is the concentration of free cupric ion; and

$K'_{CuL,Cu2+}$ is the conditional stability constant for formation of CuL complexes, expressed with respect to the free cupric ion - it is a function of pH, ionic strength, and extent of competing complexation for the copper-complexing ligand by the major cations in the lake water sample.

The Cu^{2+} and CuL concentrations in each of the copper- and added-ligand - spiked titration aliquots of the sample, are related to the reduction peak current, i_p, of the copper-added ligand complexes, measured in each aliquot:

$$[Cu^{2+}] = i_p / (s\alpha') \quad (5)$$

$$[CuL] = Cu_T - i_p/s \quad (6)$$

s is determined from the slope of the linear part of the titration curve that occurs after the natural copper-complexing ligands in the sample are saturated with copper. α' is the overall side reaction coefficient of copper:

$$\alpha' = \alpha_{Cu'} + \alpha_{CuAL} \quad (7)$$

where $\alpha_{Cu'}$ is the inorganic side reaction coefficient for copper, which expresses the extent of interaction of Cu^{2+} with inorganic ligands. In freshwater, inorganic copper speciation is dominated by carbonate and hydroxide complexation; complexation of copper by other inorganic ligands such as SO_4^{2-} and Cl^- is negligible (Morel and Hering, 1993). Thus, the predominant inorganic copper complexes are $CuOH^+$, $Cu(OH)_2$, $CuCO_3$, and $Cu(CO_3)_2^{2-}$. $\alpha_{Cu'}$ is then calculated as follow:

$$\alpha_{Cu'} = 1 + K_{CuOH+}[OH^-] + \beta_{Cu(OH)2}[OH^-]^2 + K_{CuCO3}[CO_3^{2-}] + \beta_{Cu(CO3)2}[CO_3^{2-}]^2 \quad (8)$$

α_{CuAL} is the side reaction coefficient for complexation of copper by added ligand, AL; its value is controlled by the concentration of AL in the sample:

$$\alpha_{CuAL} = 1 + \Sigma \beta_{Cu(AL)n,Cu2+}[AL']^n \quad (9)$$

where $ß_{Cu(AL),Cu2+}$ is the conditional stability constant of each of the CuAL complexes formed in the sample at fixed pH, salinity, and ionic strength; and [AL'] is the concentration of AL not complexed by copper. In virtually all cases for natural waters, [AL'] is always greater than Cu_T; thus, [AL'] essentially equals the total analytical concentration of AL (i.e., C_{AL}).

Values for α_{CuAL}, where AL is salicylaldoxime, had to be determined experimentally because no literature values for $ß_{Cu(AL)n,Cu2+}$ exist for its copper complexes. In this study, α_{CuSA} was determined by calibration against the well known copper chelating agent, EDTA, as described by Campos and van den Berg (1994). Values for α_{CuSA} are calculated from:

$$\alpha_{CuSA} = (\alpha_{Cu'} + \alpha_{CuEDTA})X - \alpha_{Cu'}/ (1-X) \qquad (10)$$

where X is the ratio of the peak CSV reduction currents of the Cu-SA complexes measured in the presence (i_p) and in the absence (i_{po}) of EDTA:

$$X = i_p/ i_{po} \qquad (11)$$

α_{CuEDTA} is the side reaction coefficient of copper complexed with EDTA:

$$\alpha_{CuEDTA} = 1 + K'_{CuEDTA}[EDTA'] \qquad (12)$$

In the presence of one natural copper-complexing ligand, equation (2) will yield a straight line whose slope equals $1/C_L$ and whose Y-intercept equals $1/(K'_{CuL,Cu2+}C_L)$.

In the presence of two complexing ligands in the sample, the plot $[Cu^{2+}]/ [CuL]$ vs $[Cu^{2+}]$ gives a curve, and concentrations of copper-organic complexing ligands $[L_1]$ and $[L_2]$ have to be calculated separately. In this two-ligand case such plot ([Cu2+]/[CuL] vs $[Cu^{2+}]$) gives a straight line at low copper concentrations, where strong ligands complexes with strong ligands (L_1) are formed. Using the mass balance equation:

$$C_{L1} = [L'_1] + [CuL_1] \qquad (13)$$

the equilibrium expression for formation of CuL_1:

$$K'_{CuL1,Cu2+} = [CuL_1]/ [Cu^{2+}] [L'_1] \qquad (14)$$

and equation (2), the contribution of $[CuL_1]$ to $[CuL_x]$ and $K'_{CuL1,Cu2+}$ are estimated. Then, the appropriate expression ($[L'_1]$ from equation 13) is substituted in order to get the concentration of the ligand:

$$[CuL_1] = (K'_{CuL1}.[Cu^{2+}] [CuL_1])/ (1 + K'_{CuL1} [Cu^{2+}]) \qquad (15)$$

At higher concentrations of cupric ions, $[CuL_2]$ is estimated from:

$$[CuL_2] = Cu_T - [CuL_1] - i_p/ s \qquad (16)$$

A plot of $i_p/ (s[CuL_2])$ vs i_p/ s yields the first estimates for C_{L2} and K'_{CuL2}. With these values, concentrations of CuL_1 (at low cupric ion concentrations) are corrected for the

contribution of CuL_2 by using expression (17):

$$[CuL_1] = Cu_T + [CuL_2] - i_p / s \qquad (17)$$

and corrected values for K'_{CuL1}, Cu^{2+} and C_{L1} are calculated. Several iterations of this procedure give optimized values for C_{L1}, C_{L2}, $K'_{CuL1,Cu2+}$ and $K'_{CuL2,Cu2+}$.

The detection window of LC/DPCSV can be changed by changing the concentration of the added ligand in order to compete with the complexation of the study metal. This detection window is adjusted by the relative magnitudes of the side reaction coefficients for complexation of copper with added ligand (α_{CuAL}) and with natural ligand (α_{CuL}) (van den Berg et al., 1990; Donat and van den Berg, 1992). Values for the concentration of the natural copper-complexing organic ligands (C_L) in the sample, and the conditional stability constants of their copper complexes with respect to free copper ions, $K'_{CuL,Cu2+}$, are calculated from a plot of $[Cu^{2+}]/ [CuL]$ vs. $[Cu^{2+}]$ for the sample titration aliquot set. These values of C_L and K'_{CuL} are used to calculate the original concentrations of free Cu^{2+}, inorganic copper, and copper complexed with natural organic ligands (CuL) in the sample.

III. METHODS AND MATERIALS

3.1 Instrumentation

The instrumentation for LC/DPCSV used in this study consisted of an EG & G Model 303A hanging mercury drop electrode (HMDE) connected to an EG & G Model 264 voltammetric analyzer with a X-Y recorder. The reference electrode was Ag/ sat. AgCl, KCl, and the counter electrode was a platinum wire. Samples were contained in FEP-Teflon voltammetric cell cups, and stirred with a PTFE-Teflon coated stirring bar by a magnetic stirrer (EG & G Model 305).

3.2 Reagents

For total dissolved copper, 30 mL of a 1.02×10^{-2} M stock solution of salicylaldoxime, SA, (Hydroxybenzaldehyde oxime, $C_7H_7NO_2$) (Sigma Chemical Co.) was prepared in 9.8×10^{-2} M HCl using Milli-Q water (Millipore, resistivity equal to 18 M Ω cm^{-1}). The SA concentration in 10 mL of lake water sample was 2.53×10^{-5} M. Even though Campos and van den Berg (1994) claimed that this SA stock solution is stable for at least eight weeks at 4 °C, new stock solutions were prepared each time. The buffer of pH 8.48 (30 mL) was prepared using 1 M boric acid (GFS Chemicals) and 0.35 M ammonium hydroxide. The HEPES buffer solution [4-(2-hydroxyethyl)-1-piperazine ethane-sulfonic acid] (Aldrich Chemical Company, Inc.) was 1.012 M HEPES and

0.598 M ammonium hydroxide (TraceMetal Grade, Fisher Scientific). Buffer solutions were found to be stable for months. A 0.1 M 8-hydroxyquinoline (8-HQ: GFS Chemicals) stock solution was prepared in 0.2 M HCl, and the concentration of 8-HQ in a 10 mL lake water sample aliquot was 7.96×10^{-6} M. Copper standard solutions were prepared by dilution of 1000 ppm standard (Fisher Scientific) with Milli-Q water and acidified to pH 3 with HCl (TraceMetal Grade, Fisher Scientific).

For speciation titrations, a 1.939 mM SA stock solution was prepared in order to give a concentration of SA in the voltammetric cell cup equal to 3.87 μM.

For determination of α_{CuSA}, stock solutions of 2.05×10^{-5}, 8×10^{-5}, and 4.5×10^{-3} M EDTA (Sigma Chemical CO.) were prepared in Milli-Q water a day before use, and lake water samples (LP-2 and WB-3) were UV-photooxidized to remove natural complexing organic ligands.

3.3 Sampling

Water samples were collected at 2 m depth at four stations in Lake Western Branch and three stations in Lake Prince (Figures 3 and 4) during different seasons (September 1994, March, April and July 1995) to allow examination of spatial and temporal variability of copper complexation and speciation in these lakes. Water samples were collected using a Teflon tubing/ peristaltic pumping system, and filtered in-line through a 0.45 μm polypropylene cartridge filter into high-density polyethylene (HPDE) bottles (for total dissolved copper) and fluorinated linear polyethylene (FLPE) bottles for

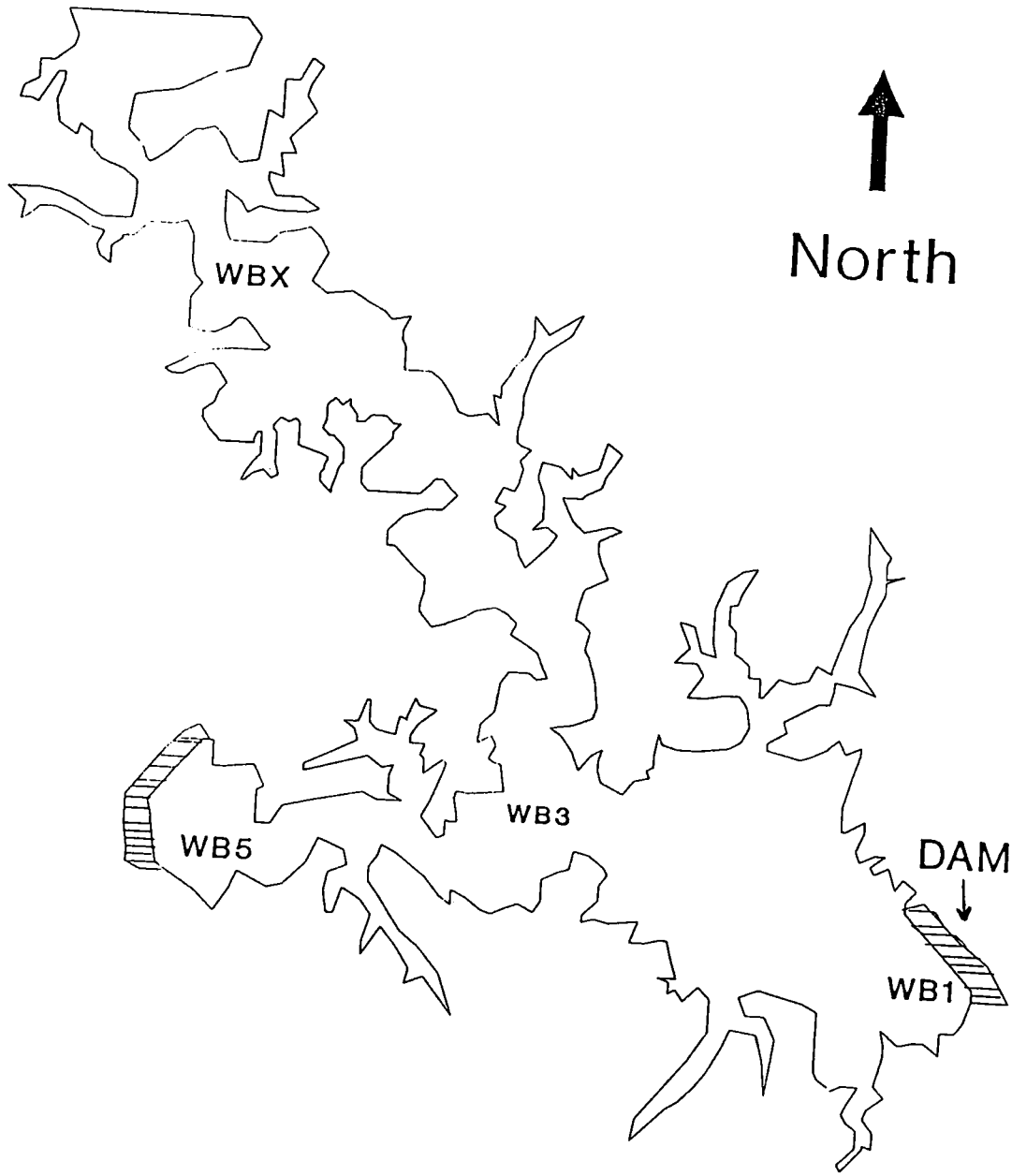


Figure 3. Lake Western Branch station locations.

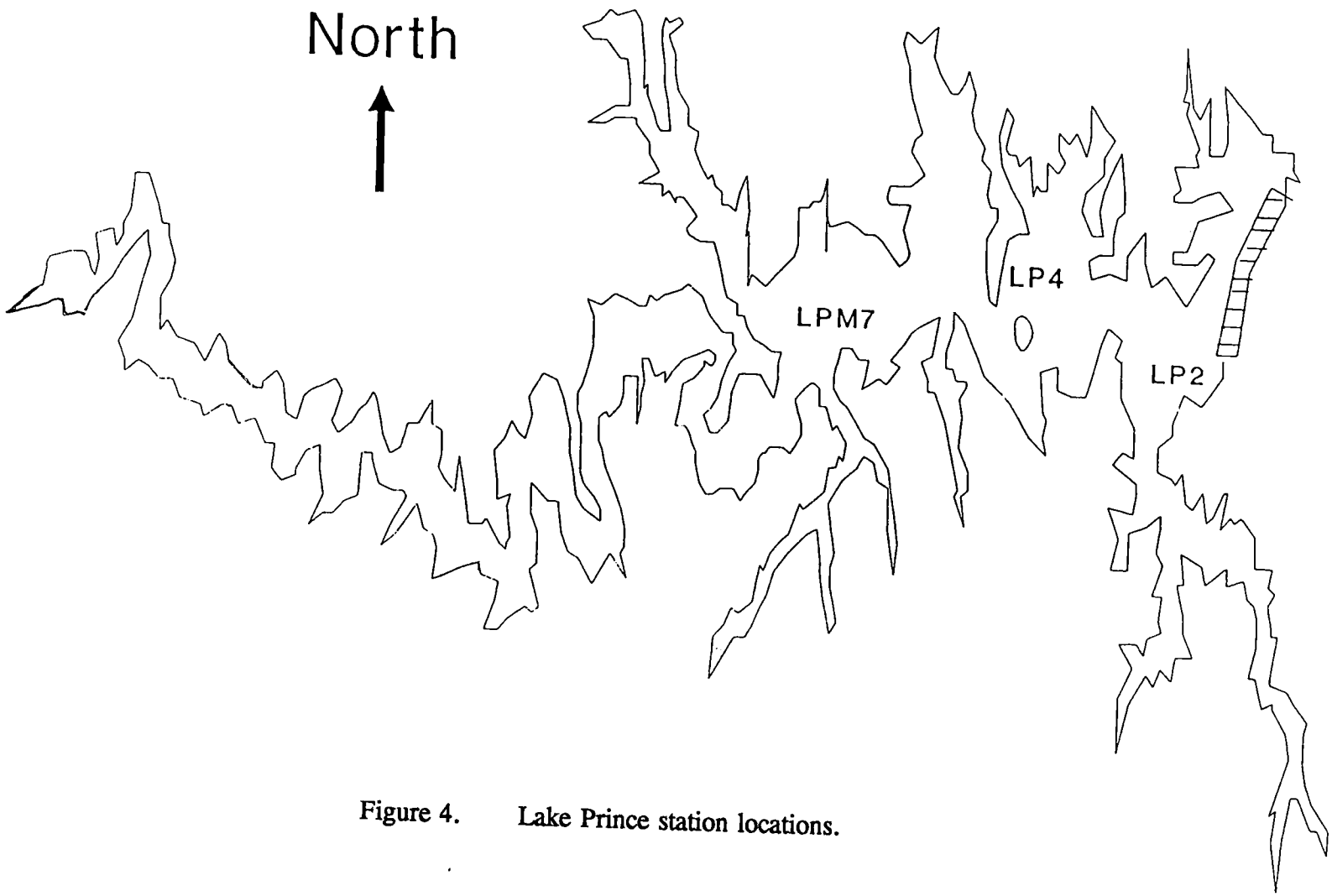


Figure 4. Lake Prince station locations.

speciation analysis. In this system, water samples contacted only rigorously acid-cleaned Teflon or other plastic surfaces. Sample bottles were carefully cleaned with ethanol, acetone, and Micro^{R} detergent followed by soaking one week in 2 M HCl and one week in 2 M HNO_3, with rinses with DI water between soaks. Finally, the bottles were rinsed with Milli-Q water and filled with pH 2 Milli-Q water/TraceMetal Grade HCl and double-bagged with ziplock plastic bags until the day of collection. Clean disposable plastic gloves were worn during all stages of sampling and laboratory analysis.

Filtered lake water samples for both total dissolved copper and speciation were double-bagged in plastic ziplock bags and returned to our research laboratory at ODU. These procedures are well-established to allow collection of uncontaminated water samples for determinations of trace metal concentrations in open ocean waters (Martin et al., 1976; Bruland et al., 1979; 1985; Donat et al., 1994; Donat, 1994), which are much lower than those likely to occur in the study lakes. The pumping system has been proven to be successful at collecting clean, uncontaminated samples for determinations of trace metal concentrations in San Francisco Bay (Flegal et al., 1991; Donat et al., 1994) and Chesapeake Bay (Donat, 1994), which are comparable to those in the study lakes. Water samples for total dissolved copper determinations were acidified to pH 2 using HCl (Optima TraceMetal Grade, Fisher Scientific), and copper speciation samples were kept cold and dark until analysis. All sample manipulations were carried out inside of a special work area supplied with double-HEPA-filtered air.

3.4 Analysis

3.4.1 Total dissolved copper determinations

Total copper was determined by DPCSV using salicylaldoxime as the adsorptive ligand and using the method of standard additions, according to the procedure described by Campos and van den Berg (1994). In this method the acidified lake water samples were transferred into acid-cleaned Teflon beakers and UV-photooxidized for five hours by a 1.2 KW Hg-arc lamp to destroy dissolved organic, copper-complexing ligands in the samples that would otherwise interfere with the analysis. After UV oxidation, the sample pH was adjusted near 7 with ultrapure ammonium hydroxide (Optima TraceMetal Grade, Fischer Scientific). Then, 10 mL of sample was pipetted into an acid-cleaned Teflon cell cup and buffered to pH 7.51 with 100 μL of HEPES buffer (0.01 M in cup) before 20 μL of 8-hydroxyquinoline solution was added, yielding an 8-HQ concentration in the sample of 7.95×10^{-6} M. Determinations were performed by mounting the cell cup to the EG & G Model 303A voltammetric analyzer, and purging the sample with ultrapure nitrogen for 4 min while stirring, to eliminate oxygen that would otherwise interfere with the analysis.

Copper was deposited at the HMDE at -1.10 V for 60 sec. The scanning parameters were 25 mV pulse height, 5 mV/ sec. scan rate, 30 sec. quiescent time. The reduction peak current for copper appeared at approximately -0.34 V.

For blank determinations, 10 mL of Milli-Q water was pipetted into a cell cup and buffered with 100 μL HEPES pH 7.48 (concentration in cup equal to 0.01 M). Then, 20 μL of 8-HQ was added (to give 7.96×10^{-6} M in cup) and cell was mounted to the HMDE. Determinations were performed by the DPCSV technique as described above.

The HEPES buffer solution was found to contribute a copper blank of 0.432 ± 0.006 nM per 100 μL of buffer solution added to a sample aliquot (i.e. the usual volume of buffer solution used). Detection limit (DL): 3s was therefore 0.018 nM.

Standard reference materials (SRM), (river water (SLRS-2), estuarine water (SLEW-1), and open ocean seawater (NASS-3) (from the National Research Board, Canada), were analyzed to ensure the reliability of the technique. The results (Table 1) are in the excellent agreement with the accepted values attesting the accuracy of the DPCSV technique.

3.4.2 Copper speciation determinations

A 130 mL aliquot of each lake sample was buffered to its natural pH values using either 10^{-3} M HEPES, 10^{-2} M borate, or 10^{-3} M HEPS.Then, 10 mL of buffered sample were pipetted into each of 12 FEP-Teflon voltammetric cell cups. Copper was added to all but one cup in this series, giving concentrations of added copper ranging from zero to 191 nM. These cell cups were placed in polyethylene containers fitted with airtight lids, and the added copper was allowed to equilibrate for 6-8 hours. Then, 20 μL of SA was spiked into each cup to give a concentration of 3.87 μM. Aliquots were again covered with airtight lids, and allowed to equilibrate overnight at room temperature.

The next day, copper complexed by SA in each sample aliquot was determined by DPCSV. Each aliquot was deaerated with ultrapure N_2 for 4 min, and copper-SA complexes were adsorbed onto the HMDE at -0.10 V for 60 seconds using slow stirring

Table 1. DPCSV determinations of total dissolved copper in standard reference materials.

Sample	Measured concentration (nM) (n = 3)	RSD (%)	Certified * concentration (nM)
SLRS-2	43.01 ± 0.19	0.44	43.43 ± 2.67
SLEW	28.10 ± 0.64	0.02	27.70 ± 1.42
NASS-3	1.59 ± 0.11	7.00	1.72 ± 0.17

* Concentrations reported at 90 % confidence interval (i.e., ± 2s of mean)

Note: SLRS-2 is river water.

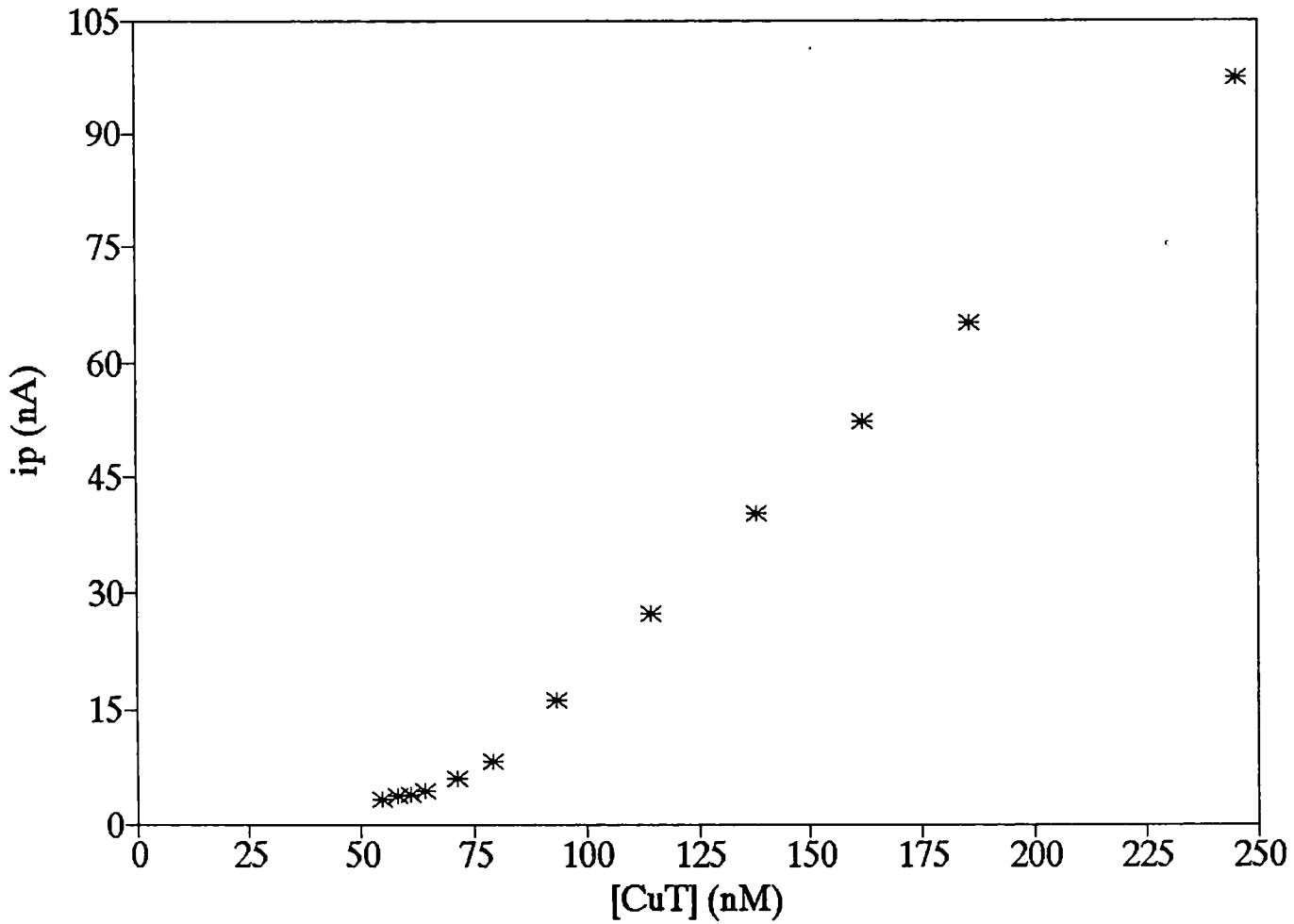
SLEW is estuarine water.

NASS-3 is open ocean water.

(400 rpm). At the end of the 60-second adsorption period, the stirrer was stopped, and 30 seconds later, the potential on the HMDE was scanned to more negative values causing reduction of Cu (II) in the Cu-SA complexes. The scanning parameters were: scan rate 5 mV/ sec., pulse height 25 mV and 5 pulses per second. The current resulting from reduction of the copper complexed by SA was recorded as a function of applied potential, producing a voltammogram.

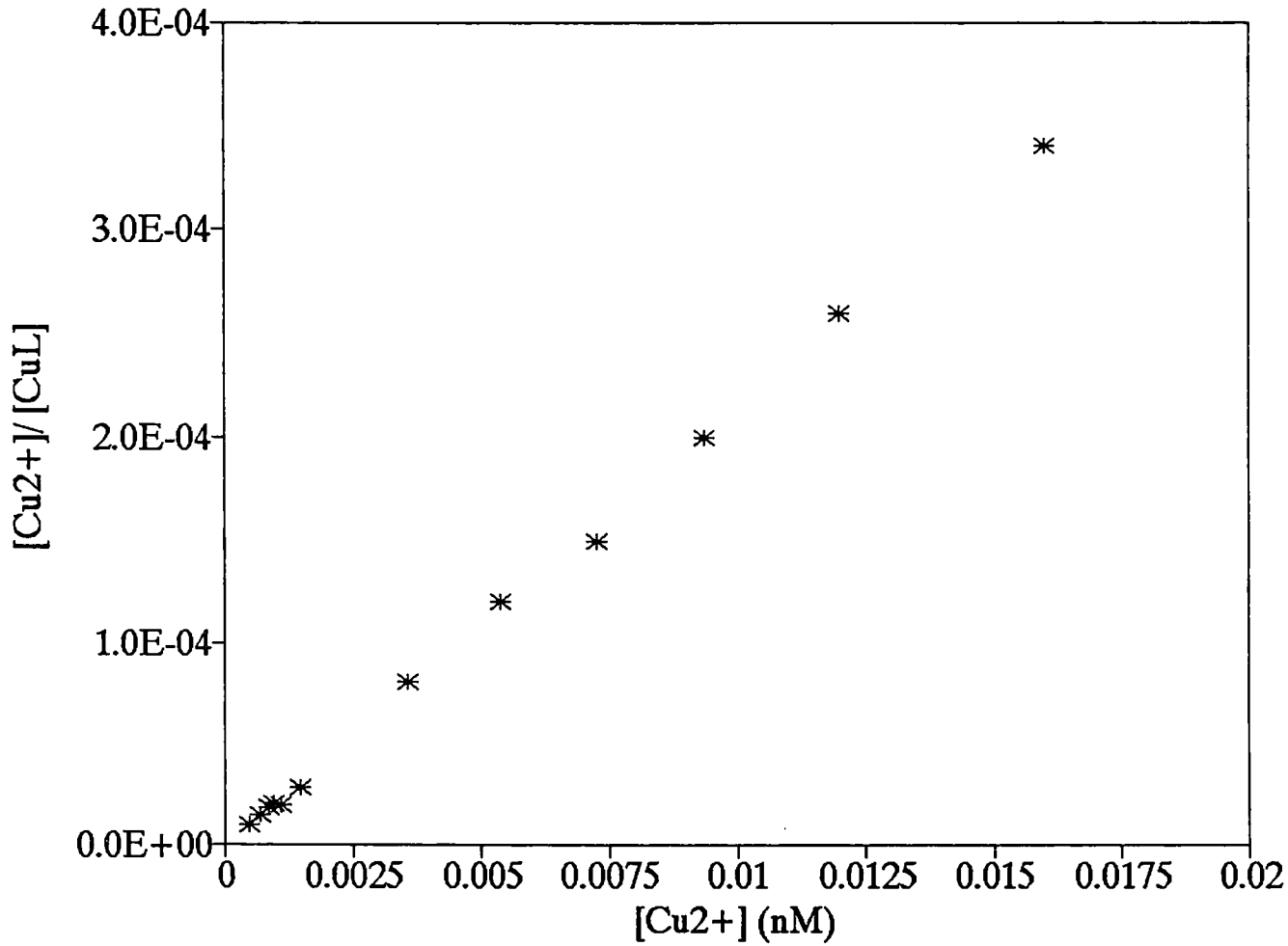
The height of the reduction peak for each aliquot was then measured from the voltammograms with a millimeter ruler, and converted to current (nA) from the XY recorder and voltammetric analyzer full scale settings. The peak currents were then plotted as a function of the total copper concentration (natural ambient + added) in each titration aliquot to produce a titration curve (e.g., see Figs. 3a, c).

The titration curve data were linearized using equation 2. Values of $[Cu^{2+}]$ and [CuL] in each titration aliquot were calculated using equations 5 and 6. Then, $[Cu^{2+}]$/ [CuL] values for each titration aliquot were plotted versus $[Cu^{2+}]$ for each titration aliquot producing a linearization plot (e.g., see Figs. 3b, 3d). If the linearization plot yielded a straight line (e.g., Fig. 3b), the sample contained a single copper-complexing ligand whose concentration was obtained from the slope of the best fit line through the linearization data, obtained by linear least-squares regression ($m = 1/ C_L$), and whose conditional stability constant was obtained from the Y-intercept $\{b = 1/ (K'C_L)\}$. If the linearization plot yielded a curve (e.g. Fig. 3d), then the presence of at least two ligands was indicated, and the procedure described on pages 19 to 20 was used to calculate C_{L1}, C_{L2}, K'_{CuL1} and K'_{CuL2}.



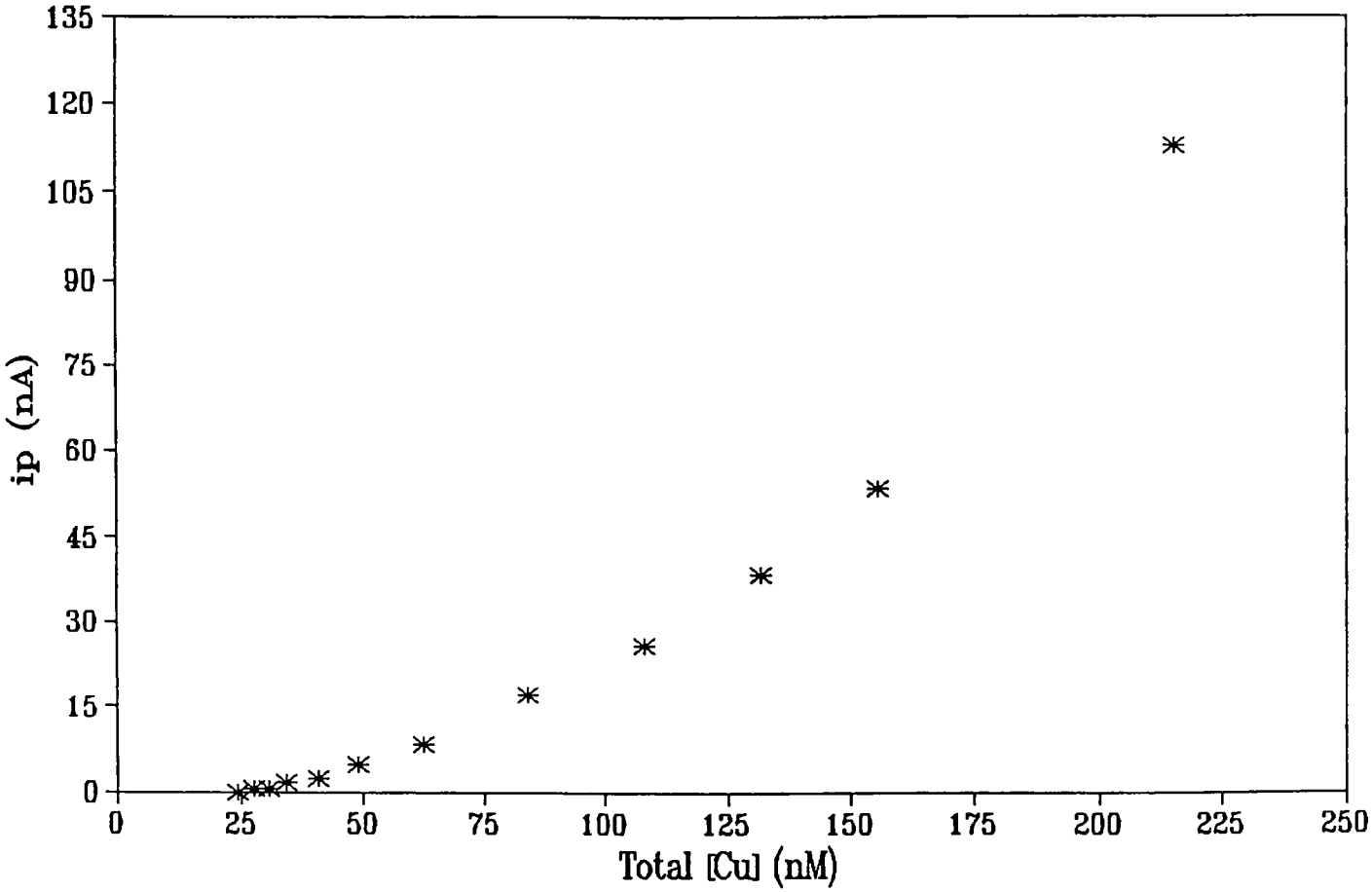
(a) Copper titration curve

station: WB-X, July 1995



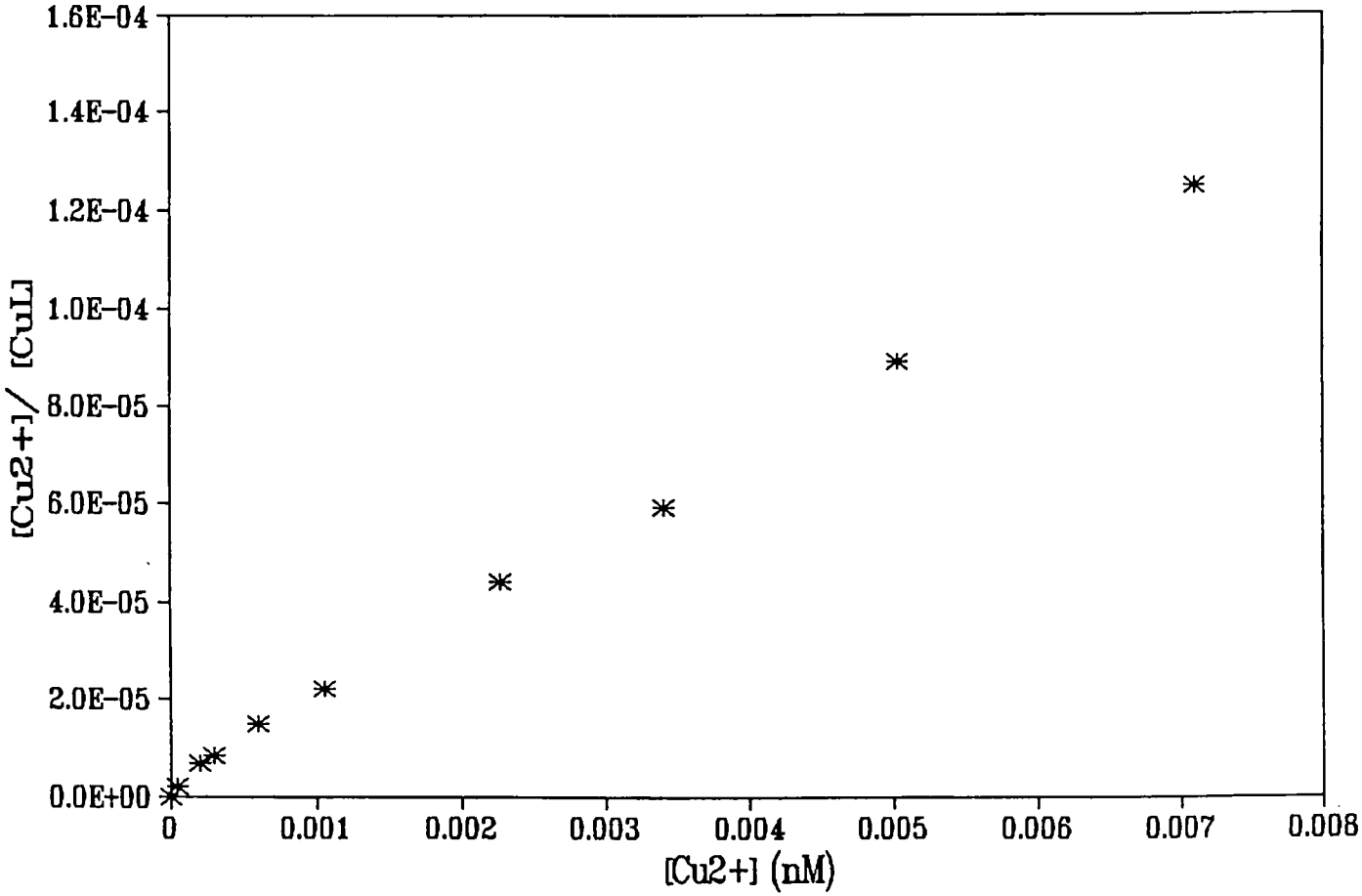
(b) Titration curve linearization

station: WB-X, July 1995



(c) Copper titration curve

station: WB-X, April 1995



(d) Titration curve linearization

station: WB-X, April 1995

3.4.3 Determination of α_{CuSA}

Values of α_{CuSA} were determined by calibration against EDTA added to the lake water samples using equations 8, 9, 10, 11, and 12.

CuSA reduction peak currents in the presence (i_p) and absence (i_{po}) of EDTA were measured using the following procedure: 190 mL of each of two lake water samples (Western Branch, WB-3; and Lake Prince, LP-2) were transferred into acid-cleaned Teflon beakers and UV-irradiated for five hours. Borate buffer solutions were added (100 μL gave 10^{-2} M concentrations in cell cup) to set the pH of the samples at their natural value (WB-3: pH 8.41, and LP-2: pH 7.48). Copper was added to both samples to yield about 30 nM, and salicylaldoxime to yield 3.87 μM. 10 mL aliquots were then pipetted into 9 FEP-Teflon cell cups, and EDTA was added to six of the nine cups in successively-increasing concentrations ranging from 3.2×10^{-7} to 2.01×10^{-8} M. Cells were placed in polyethylene containers filled with airtight lids, and allowed to equilibrate overnight (10 hours) at room temperature. Copper complexed by salicylaldoxime (SA) was determined by DPCSV following the procedure described elsewhere (section 3.4.2).

In freshwater, inorganic copper speciation is dominated by CO_3^{2-} ($10^{-5.82}$ M) and OH^- ($10^{-6.524}$ M); copper complexation by other inorganic ligands such as SO_4^{2-} and Cl^- is negligible (Morel and Hering, 1993). Thus, the predominant inorganic copper complexes are $CuOH^+$, $Cu(OH)_2$, $CuCO_3$ and $Cu(CO_3)_2^{2-}$. Using equation 8 above, the value for $\alpha_{Cu'}$ in Lakes Western Branch and Prince is calculated to be 9.624, using values for stability constants from Morel and Hering (1993).

Values for K'_{CuEDTA} (at 25 °C and μ = 0) and other EDTA-complexes formed were calculated using the Titrator program for chemical equilibrium calculations

(Cabaniss, 1986). The effect of additions of EDTA on the copper reduction current in samples WB-3 and LP-2 is shown in Figure 6 and 7 respectively.

For the LP-2 sample, log $\alpha_{CuSA} = 4.365 \pm 0.198$ (n = 6); and the WB-3 sample, log $\alpha_{CuSA} = 4.116 \pm 0.442$ (n = 6); Therefore, the values of α_{CuSA} were similar in both lake samples.

α' is the sum of α_{Cu}, copper and α_{CuSA}. In practice, α' is dominated by α_{CuSA}. Therefore, from equation (7), α' values were calculated to be 1.31×10^4 for Lake Western Branch and 2.32×10^4 for Lake Prince.

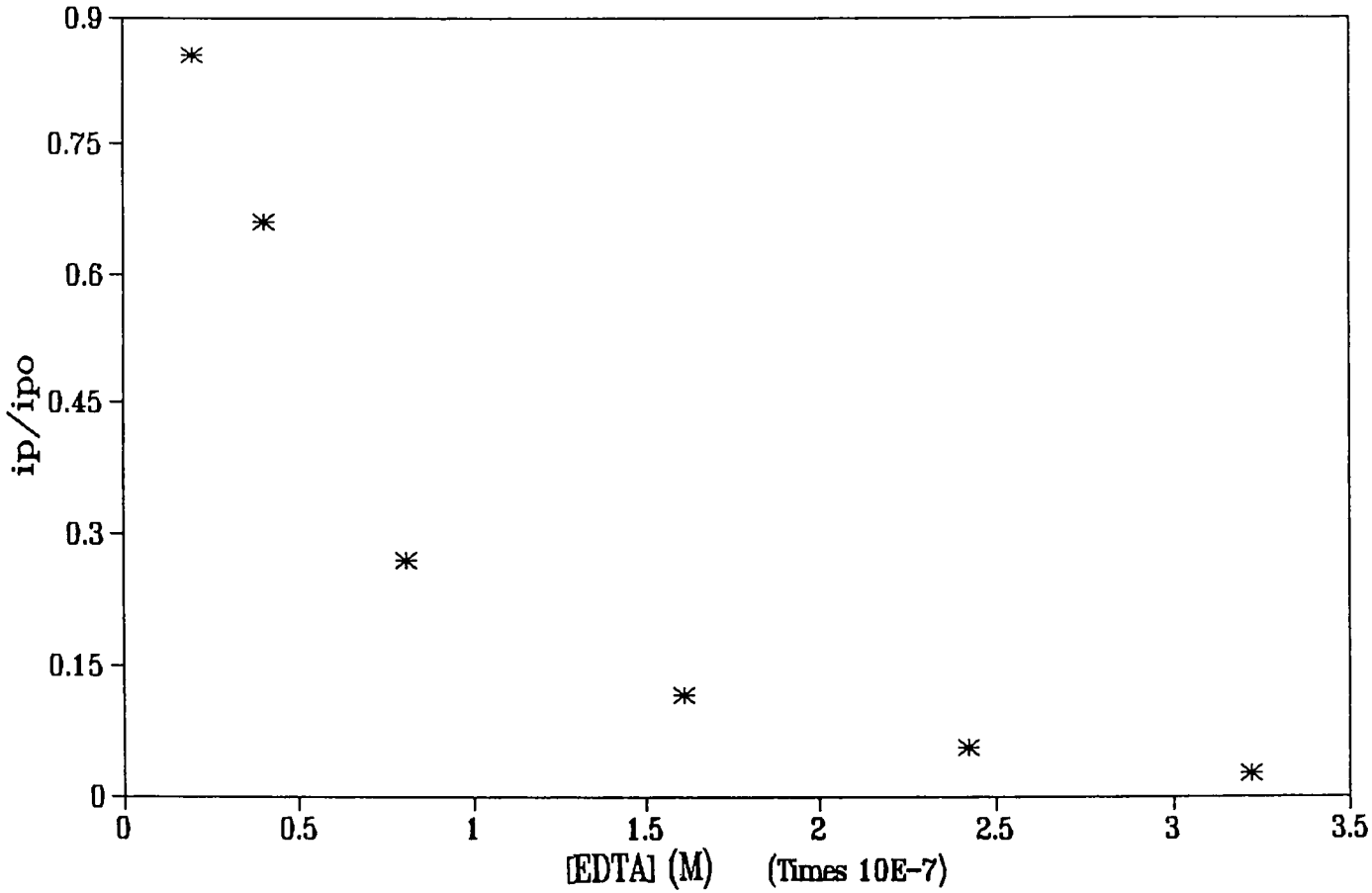


Figure 6. Sample: WB-3. Effect of EDTA on the DPCSV copper reduction current. i_p/i_{po} = X, [SA] = 3.87 μM, [Cu] = 30 nM, [Borate] = 10^{-2}M, pH 7.48.

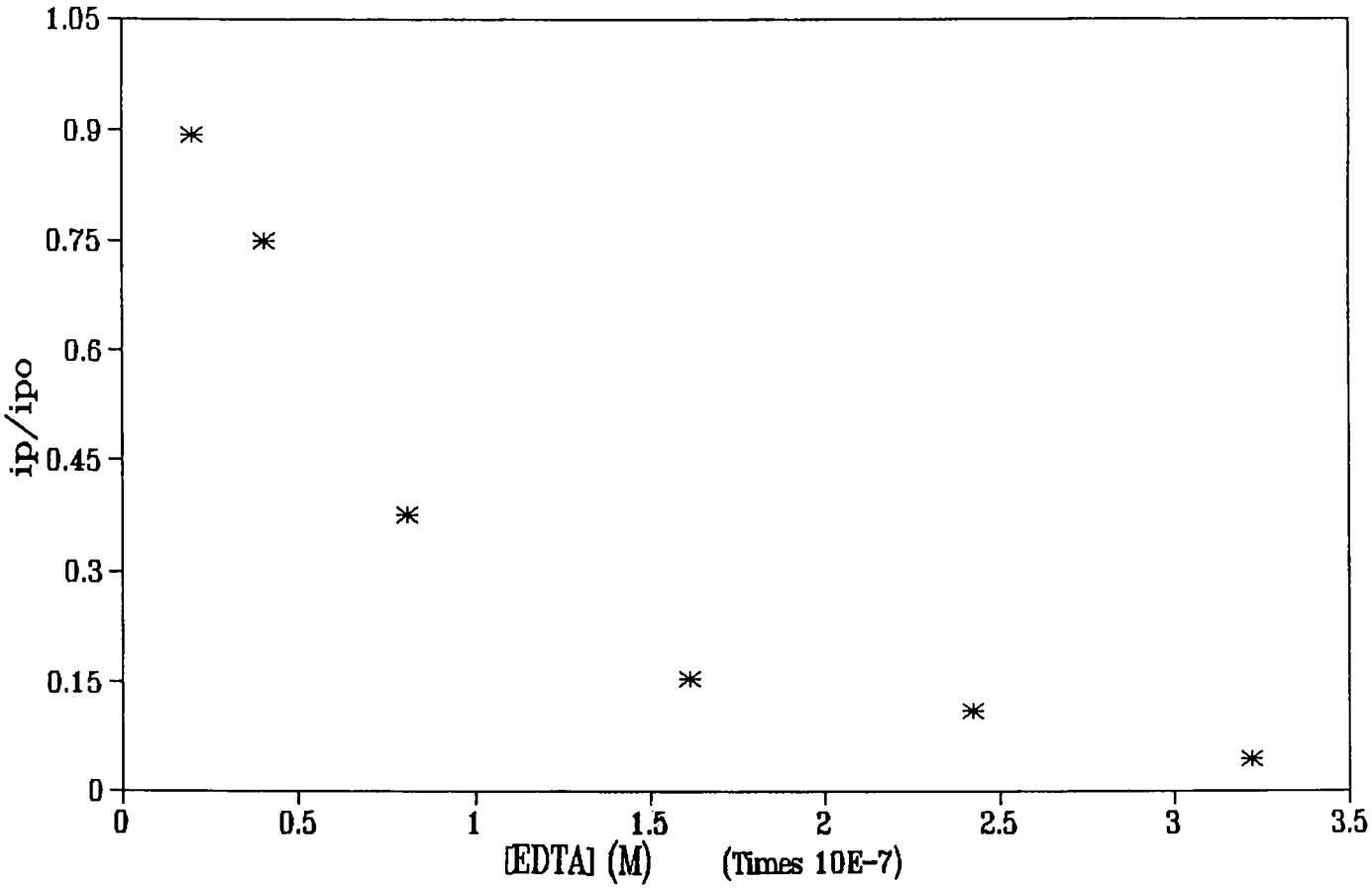


Figure 7. Sample: LP-2. Effect of EDTA on the DPCSV copper reduction current. i_p/i_{po} = X, [SA] = 3.87 μM, [Cu] = 30 nM, [Borate] = 10^{-2}, pH 7.48.

IV. RESULTS AND DISCUSSION

Only one study (Xue and Sigg, 1993) of copper complexation and speciation has been performed in a freshwater lake using LC/DPCSV. In that study, Xue and Sigg used catechol as the competing ligand. However, as explained by van den Berg and Donat (1992) and Donat et al. (1994), use of several different competing ligands, or use of different speciation methods, can yield a more complete estimation of the true speciation of a metal in a natural water sample.

Thus, because the only other freshwater lake study reported used only a single competing ligand, I wanted to attempt to study copper complexation and speciation in Lakes Western Branch and Prince using a suite of competing ligands. Of all the metals for which LC/DPCSV speciation methods have been developed, copper is the only metal for which several competing ligands have been used in LC/DPCSV measurements: catechol (van den Berg, 1984; Xue and Sigg, 1993); 8-hydroxyquinoline (van den Berg et al., 1990; Donat et al., 1994); tropolone (Donat and van den Berg, 1992); salicylaldoxime (Campos and van den Berg, 1994); and benzoylacetone (Moffett, 1995).

In my research reported here, I attempted to use catechol, 8-hydroxyquinoline, and salicylaldoxime to study copper complexation and speciation in Lakes Western Branch and Prince. The benzoylacetone method was only published in November 1995.

4.1 Comparison of various competing ligands

A comparison of the sensitivity (reduction current per nanomolar copper concentration per minute of adsorption time) for copper analysis among the three LC/DPCSV copper ligands, catechol, 8-hydroxyquinoline, and salicylaldoxime, was performed in aliquots of UV-seawater at pH 7.8 and buffered with HEPES. Samples were titrated with copper according to the procedures described in section 3.4.1, and results showed that salicylaldoxime sensitivity (2.05 $nA^{-1}nM^{-1}min^{-1}$) is almost two-fold higher than catechol (1.29 $nA^{-1}nM^{-1}min^{-1}$) and near three-fold higher than 8-hydroxyquinoline (0.8 $nA^{-1}nM^{-1}min^{-1}$). Because Xue and Sigg (1993) used catechol successfully in LC/DPCSV studies of copper complexation and speciation in Lake Greifen, a eutrophic Swiss lake, samples from Lake Western Branch and Lake Prince were initially titrated with copper in the presence of different concentrations of catechol, but the catechol concentration range recommended by Xue and Sigg (1993) (10^{-5} - 10^{-4} M) yielded no copper signal even at relatively high copper concentrations. When I extended the catechol concentration range to 10^{-7} - 10^{-3} M, I still observed no copper reduction signal. Higher concentrations of catechol were not used because of the potential for catechol out-competing the natural organic ligands for complexing copper, and the potential for saturating the surface of the HMDE with uncomplexed catechol.

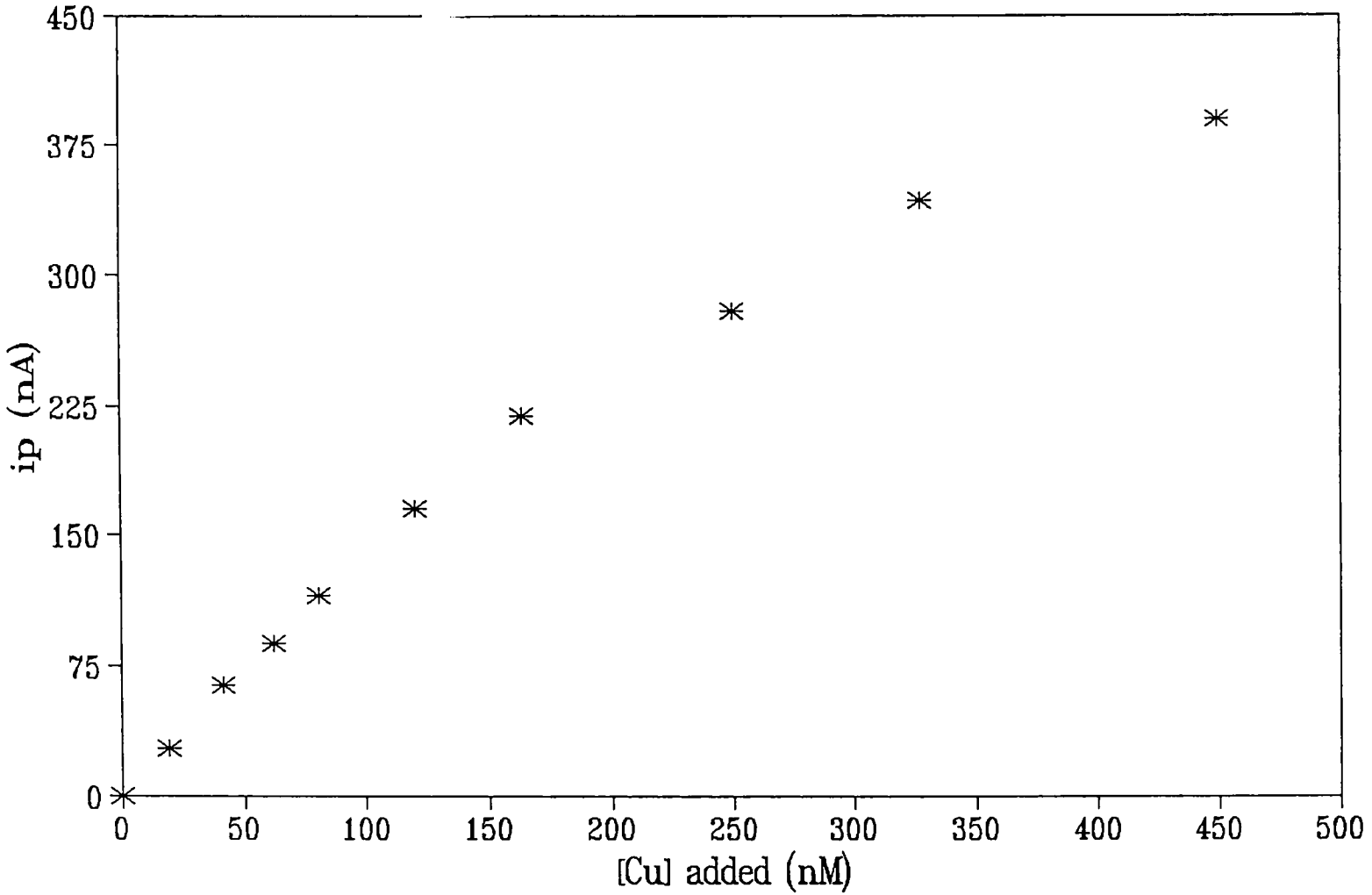
Titration experiments with 8-hydroxyquinoline (oxine) were performed by varying the concentration (0.8 - $2x10^{-5}$ M) to establish the optimal analytical conditions for copper, as described by van den Berg (1986) for seawater. However, no copper reduction signal was observed. Catechol and oxine did not produce a copper reduction current perhaps because of other interfering processes (e.g., reaction of added ligand with lake

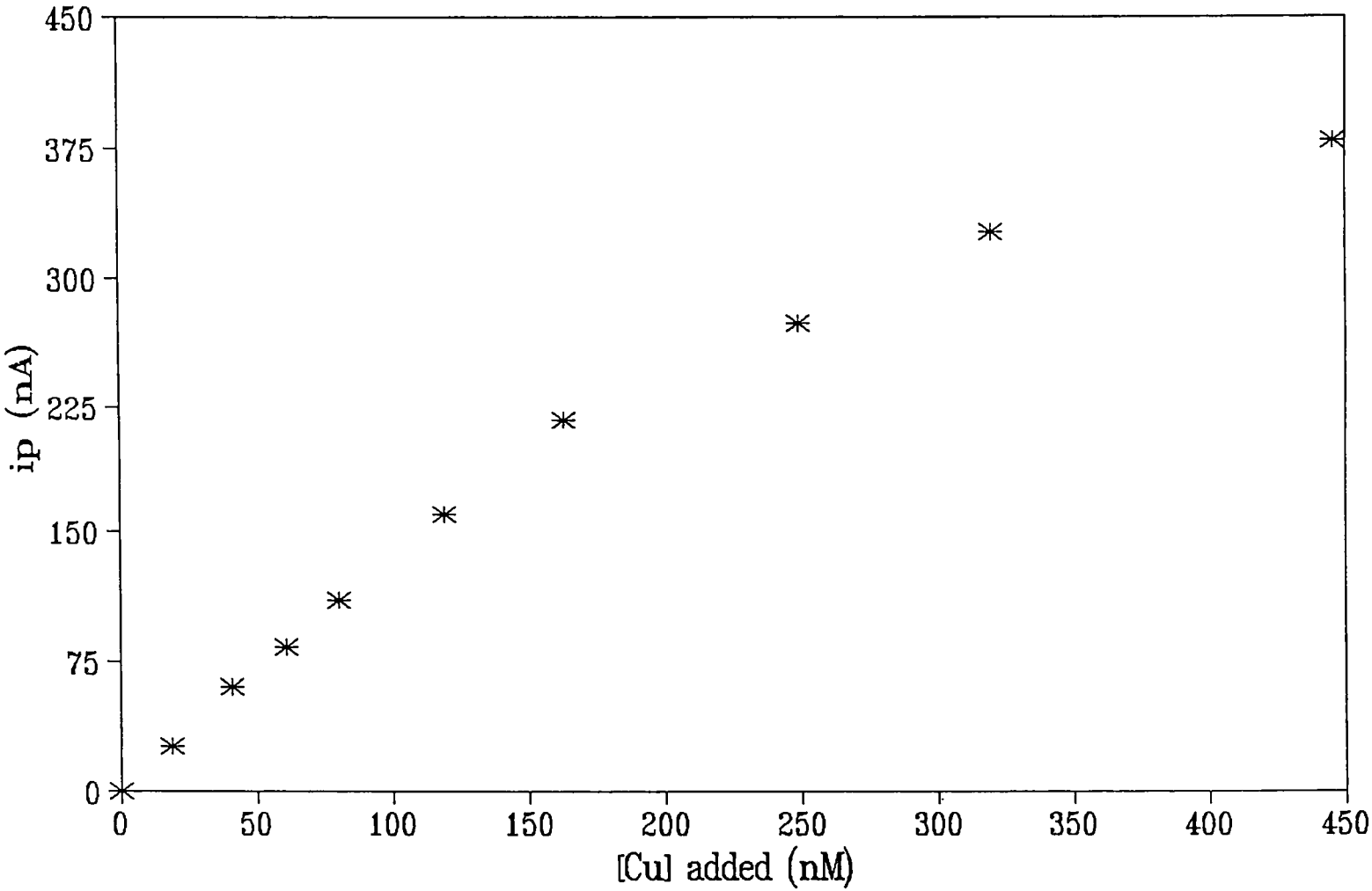
ligands).

The concentration of salicylaldoxime was varied from 1 to 10 μM in these lake water samples, and a high copper reduction peak current was observed when the concentration of SA was 3.87 μM. Beyond this concentration of SA the reduction current decreased perhaps due to saturation of the HMDE by free SA at high concentrations; therefore, salicylaldoxime was chosen as the competing ligand for LC/DPCSV titrations of the lake water samples.

4.2 Linear titration range

Figures 8 and 9 illustrate the copper reduction current obtained as a function of copper concentration in the presence of salicylaldoxime. 10 mL aliquots of a Lake Western Branch sample and a Lake Prince sample containing 3.87 μM salicylaldoxime and 0.01 M borate (pH 8.48) were pipetted into 12 FEP-Teflon cups, and incrementally-increasing copper concentrations were added to each cup yielding copper concentrations ranging from 0 to 450 nM. For a deposition time of 60 s, the copper reduction current increased linearly with copper concentration to approximately 190 nM, giving a linear range sufficient for copper speciation titrations in Lakes Western Branch and Prince.


450
375
300
225
150
75
0
ip (nA)
0
50
100
150
200
250
300
350
400
450
500
[Cu] added (nM)


450
375
300
225
150
75
0
ip (nA)
0
50
100
150
200
250
300
350
400
450
[Cu] added (nM)

4.3 Total dissolved copper

The following results were obtained using the procedure described in section 3.4.1. Total dissolved copper concentrations in Lake Western Branch ranged from 7.13 to 25.53 nM in Sept/94, from 18.50 to 26.30 nM in March/95, from 24.60 to 33.24 nM in April/95, and from 54.69 to 65.25 nM in July/95. In Lake Prince total dissolved copper ranged from 5.96 to 26.98 nM in Sept/94, from 7.76 to 10.58 nM in March/95, from 10.64 nM to 12.93 nM in April/95, and from 44.5 to 59.17 nM in July/95 (see Table 2). These results indicate that copper concentrations increased almost three times in Lake WB from September 1994 to July 1995, and almost four times in LP within the same period (Figure 10). Intentional additions of copper sulfate by the City of Norfolk may contribute to the increases in the dissolved copper concentrations in both lakes. From September 1994 to July 1995 about 500 lbs of copper sulfate plus 50 gal. of Cutrin (a commercial copper chelating agent) have been regularly added to these lakes to controll blooms of algae. Other factors, including seasonal variations in rainfall, pH, and evaporation probably also contribute to variations in the dissolved copper concentrations.

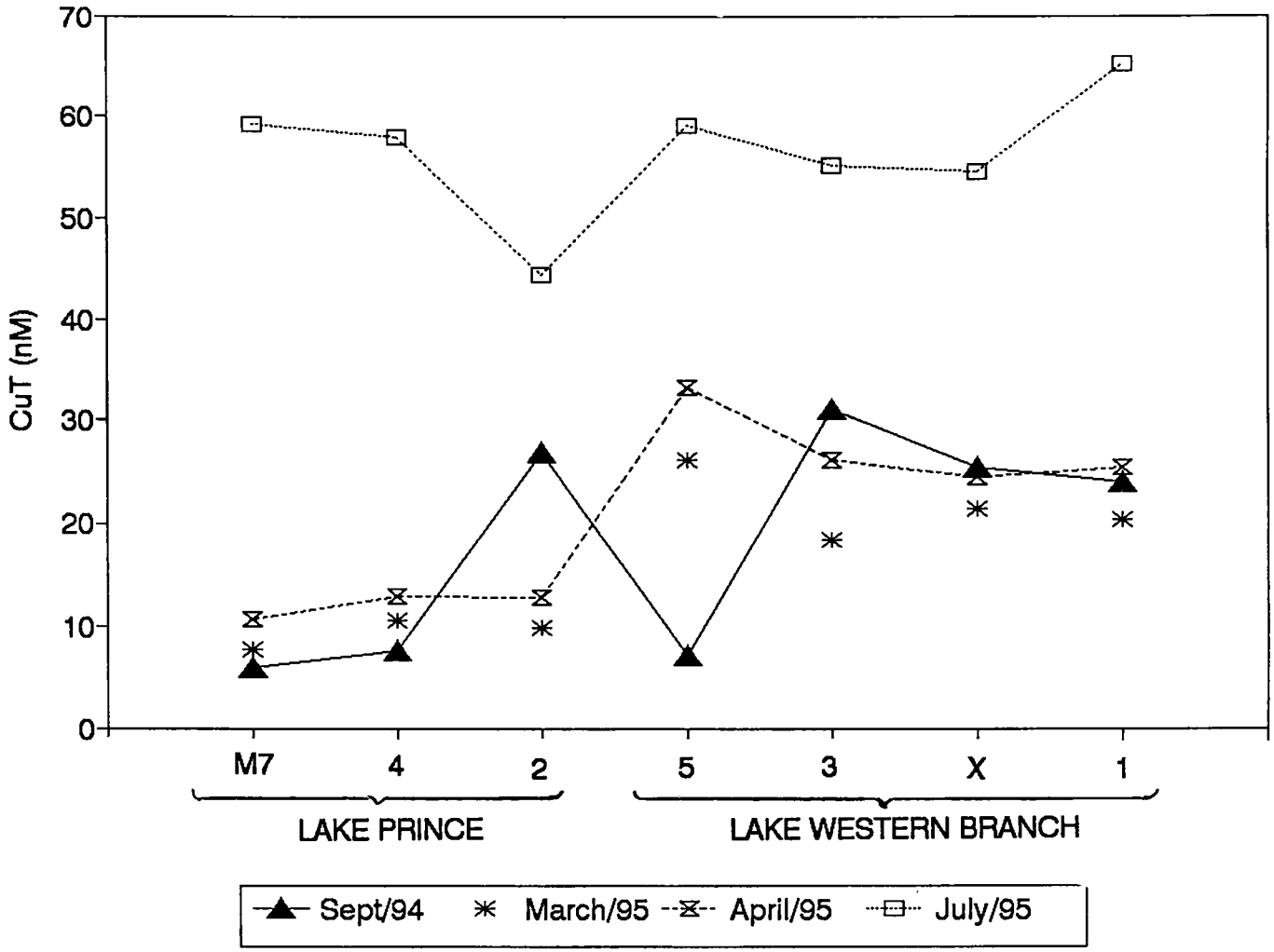
4.4 Copper-complexing ligand concentrations and conditional stability constants

The results indicated that two classes of copper-complexing ligands (L_1 and L_2) were present in both lakes in April 1995, with the L_1 class being stronger

Table 2. Temperature, pH, total dissolved copper concentrations (Cu_T), and free Cu^{2+} concentrations in Lakes Western Branch and Prince surface waters (2m depth) in Sept.'94 and March, April, and July '95.

Month/year	Station	Temp °C	pH	Cu_T (nM) (n=3)	$[Cu^{2+}]$ * (M)
Sept/94	LP-M7	25.6	6.63	5.96±0.60	$(5.37\pm0.53\times10^{-15})$
	LP-4	25.4	7.24	7.61±0.20	$(0.97\pm0.04\times10^{-15})$
	LP-2	25.3	7.44	26.98±0.5	$(93.60\pm3.20\times10^{-15})$
	WB-5	27.0	7.68	7.13±0.11	$(15.00\pm0.80\times10^{-15})$
	WB-3	26.5	7.02	31.12±0.21	(0.41±0.03)
	WB-X	26.0	7.49	25.53±0.87	(0.47±0.04)
	WB-1	26.3	7.68	24.18±0.28	$(18.00\pm1.50\times10^{-15})$
March/95	LP-M7	13.9	7.31	7.77±0.43	$(0.19\pm0.02\times10^{-15})$
	LP-4	13.6	7.30	10.58±0.33	$(0.113\pm0.006\times10^{-15})$
	LP-2	13.1	7.43	9.86±0.46	$(4.03\pm0.32\times10^{-15})$
	WB-5	13.4	6.97	26.30±0.30	$(0.55\pm0.05\times10^{-15})$
	WB-3	13.5	7.68	18.50±1.06	$(0.35\pm0.02\times10^{-15})$
	WB-X	13.3	7.49	21.54±0.45	$(9.99\pm0.05\times10^{-15})$
	WB-1	13.0	7.81	20.44±0.68	$(1.18\pm0.06\times10^{-15})$
April/95	LP-M7	17.0	7.64	10.65±0.11	$0.27\pm0.02\times10^{-15}$
	LP-4	15.7	7.46	12.93±0.68	$0.137\pm0.004\times10^{-15}$
	LP-2	16.2	7.20	12.73±0.17	$5.20\pm0.73\times10^{-15}$
	WB-5	15.3	7.41	33.24±0.59	$0.69\pm0.06\times10^{-15}$
	WB-3	14.0	8.08	26.30±0.30	$0.498\pm0.003\times10^{-15}$
	WB-X	16.2	7.29	24.60±0.77	$24.9\pm0.14\times10^{-15}$
	WB-1	17.0	7.96	25.52±0.03	$1.48\pm0.02\times10^{-15}$
July/95	LP-M7	29.8	8.10	59.17±0.28	$5.33\pm0.12\times10^{-13}$
	LP-4	29.8	7.20	57.94±0.52	$0.74\pm0.04\times10^{-13}$
	LP-2	29.8	8.10	44.50±0.53	$15.4\pm0.22\times10^{-13}$
	WB-5	30.8	6.80	59.00±1.08	$12.4\pm0.67\times10^{-13}$
	WB-3	31.1	7.50	55.20±0.91	$0.73\pm0.04\times10^{-9}$
	WB-X	30.8	6.80	54.69±0.29	$0.83\pm0.05\times10^{-9}$
	WB-1	30.0	9.00	65.25±0.74	$4.65\pm0.26\times10^{-13}$

* For September/94 and March/95 Cu^{2+} concentrations were estimated based upon calculations of organic speciation in April/95 and July/95.


70
60
50
40
30
20
10
0
CuT (nM)
M7
4
2
5
3
X
1
LAKE PRINCE
LAKE WESTERN BRANCH
Sept/94
March/95
April/95
July/95

(log K'_{CuL1} = 13.0 to 14.7) than the L_2 class (log K'_{CuL2} = 7.3 to 8.4). However, in July 1995, both lakes showed evidence only of the stronger L_1 class (Tables 3, 4, 5, and 6).

In Lake Western Branch concentrations of L_1 ranged from 33 to 43 nM in April 1995, and from 47 to 83 nM in July 1995, while values of log K'_{CuL1} were in the range from 13.0 to 14.7 in April 1995, and from 12.4 to 13.1 in July 1995. Concentrations of the weaker ligand class (L_2) ranged from 28 to 50 nM in April 1995 with log K'_{CuL2} values ranging from 7.2 to 8.2. The L_2 ligand class was not detected in July 1995 (Tables 3 and 4).

In Lake Prince L_1 concentrations ranged from 27 to 38 nM in April 1995, and from 55 to 79 nM in July 1995. As in Lake Western Branch, log K'_{CuL1} values ranged from 13.2 to 14.6 in April 1995, and from 12.4 to 13.6 in July 1995. Concentrations of the weaker ligand class, L_2, ranged from 11 to 17 nM, and log K'_{CuL2} ranged from 7.9 to 8.4 in April 1995. The L_2 ligand class was not detected in July 1995 (Tables 5 and 6). Thus, the concentration and strengths of L_1 were similar in both Lakes Western Branch and Prince in April and July 1995. However, in both lakes, L_1 concentrations in July 1995 were almost twice as great as in April (see Figure 11).

L_1 and log K'_{CuL1} in both Lake Western Branch and Lake Prince are comparable to those determined by Xue and Sigg (1993) in Lake Greifen, an eutrophic Swiss lake (L_1: 40 to 88 nM, and log K'_{CuL1}: 13.9 to 14.9), using LC/DPCSV with catechol. However, the concentrations and strengths of the weaker ligand class, (L_2), in Lake Western Branch and Lake Prince are lower than those observed reported by Xue and Sigg (1993) in Lake Greifen (C_{L2}: 254 to 556 nM; log K'_{CuL2}: 11.8 to 12.9). The speciation of dissolved copper in Lakes Western Branch and Prince was dominated by (L_1) complexes which account for >99 % of total dissolved copper, consistent with the

Table 3. Copper-complexing ligand concentrations and conditional stability constants, and copper speciation in surface waters (2m depth) of Lake Western Branch - April 1995 (n = 2).

	Stations			
	WB-5	WB-3	WB-X	WB-1
C_{L1} (nM)	42.99±0.99	42.02±0.13	42.51±0.20	33.33±0.10
C_{L2} (nM)	28.95±0.23	27.77±0.26	34.74±0.53	49.72±0.20
log K'_{CuL1}	13.99±0.71	14.52±0.07	14.71±0.03	13.05±0.10
log K'_{CuL2}	7.27±0.08	7.29±0.08	7.52±0.01	8.25±0.30
Cu_T (nM)	33.24±0.59	26.30±0.30	24.60±0.77	25.52±0.03
CuL_1 (nM)	25.52±0.01	26.10±0.01	33.24±0.01	24.60±0.14
CuL_2 (pM)	$2.7\pm0.2\times10^{-2}$	$2.2\pm0.1\times10^{-3}$	$8.4\pm1.1\times10^{-3}$	2.19±0.16
Cu' (pM) *	0.97±0.22	250.0±10.0	1.50±0.71	0.81±0.32
Cu^{2+} (fM) **	6.9±0.6	4.98±0.03	249.0±1.4	14.8±0.2

* pM = 10^{-12}

** fM = 10^{-15}

Table 4. Copper-complexing ligand concentrations and conditional stability constants, and copper speciation in surface waters (2m depth) of Lake Western Branch - July 1995 (n = 2).

	Stations			
	WB-5	WB-3	WB-X	WB-1
C_{L1} (nM)	62.80±0.99	55.21±1.10	46.71±0.45	83.44±0.05
log K'_{CuL1}	13.06±0.55	12.45±0.37	13.07±0.84	12.87±0.09
Cu_T (nM)	59.00±1.08	55.20±0.91	54.69±0.29	65.25±0.74
CuL_1 (nM)	53.42±0.25	53.57±0.18	51.53±0.17	64.32±0.13
Cu' (pM)	0.36±0.05	0.11±0.02	7.98±0.02	0.07±0.01
Cu^{2+} (pM)	1.24±0.07	730±40	830±50	0.46±0.03

Table 5. Copper-complexing ligand concentrations and conditional stability constants, and copper speciation in surface waters (2m depth) of Lake Prince - April 1995 (n = 2).

	Stations		
	LP-M7	LP-4	LP-2
C_{L1} (nM)	33.73±0.98	37.53±0.11	26.71±0.96
C_{L2} (nM)	10.81±0.48	16.56±0.44	N.D.
log K'_{CuL1}	14.23±0.10	14.58±0.04	13.24±0.20
log K'_{CuL2}	7.98±0.76	8.35±0.29	N.D.
Cu_T (nM)	10.65±0.11	12.93±0.68	12.73±0.17
CuL_1 (nM)	10.64±0.01	12.74±0.26	12.34±0.24
CuL_2 (nM)	$9.20\pm0.38\times10^{-7}$	$32.40\pm1.26\times10^{-7}$	N.D.
Cu' (pM)	3.00±1.26	360±140	400±240
Cu^{2+} (fM)	2.7±0.2	1.37±0.04	52.0±7.3

N.D. = "not detected"

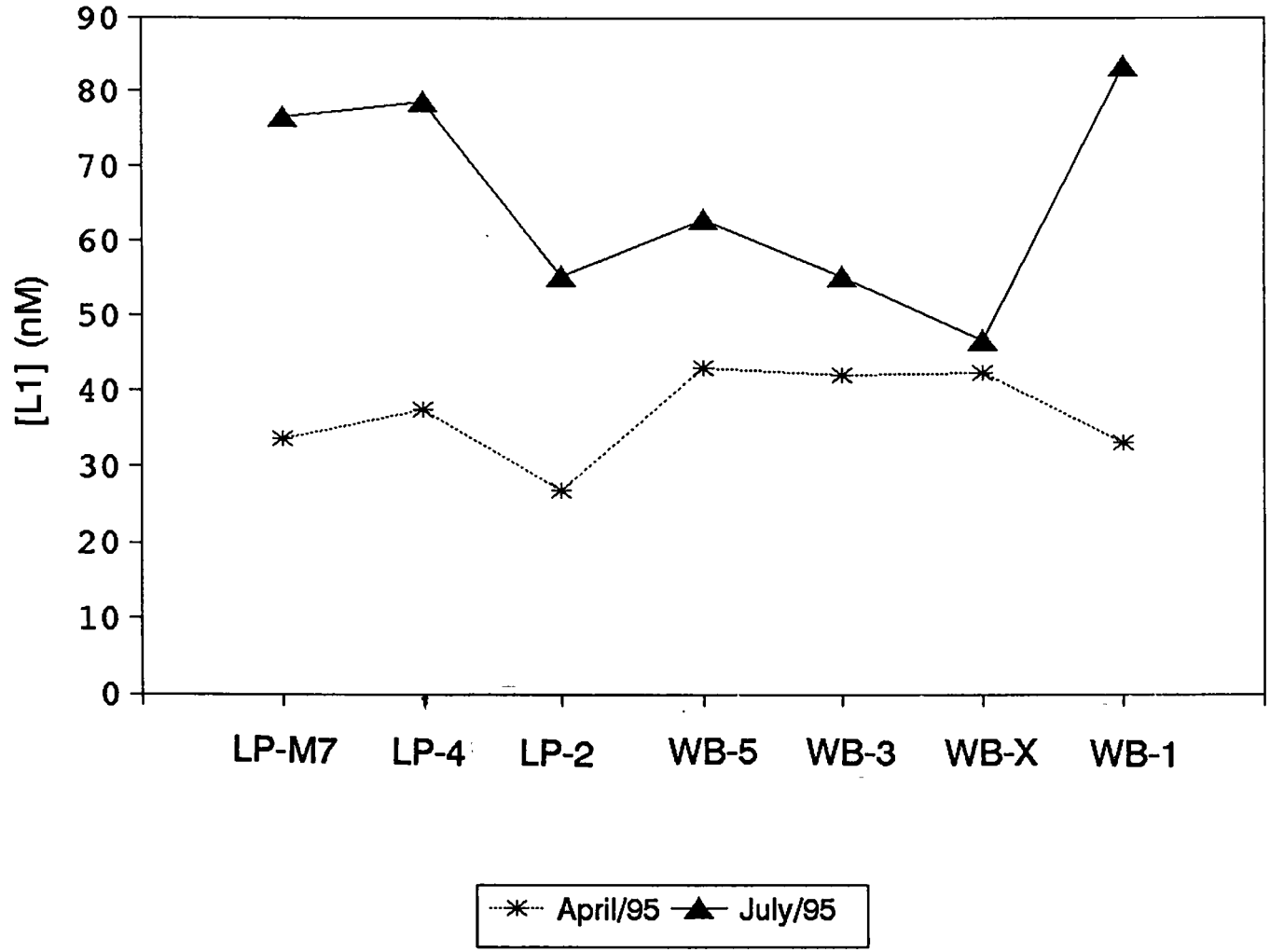
pM = 10^{-12}

fM = 10^{-15}

Table 6. Copper-complexing ligand concentrations and conditional stability constants, and copper speciation in surface waters (2m depth) of Lake Prince - July 1995 (n = 2).

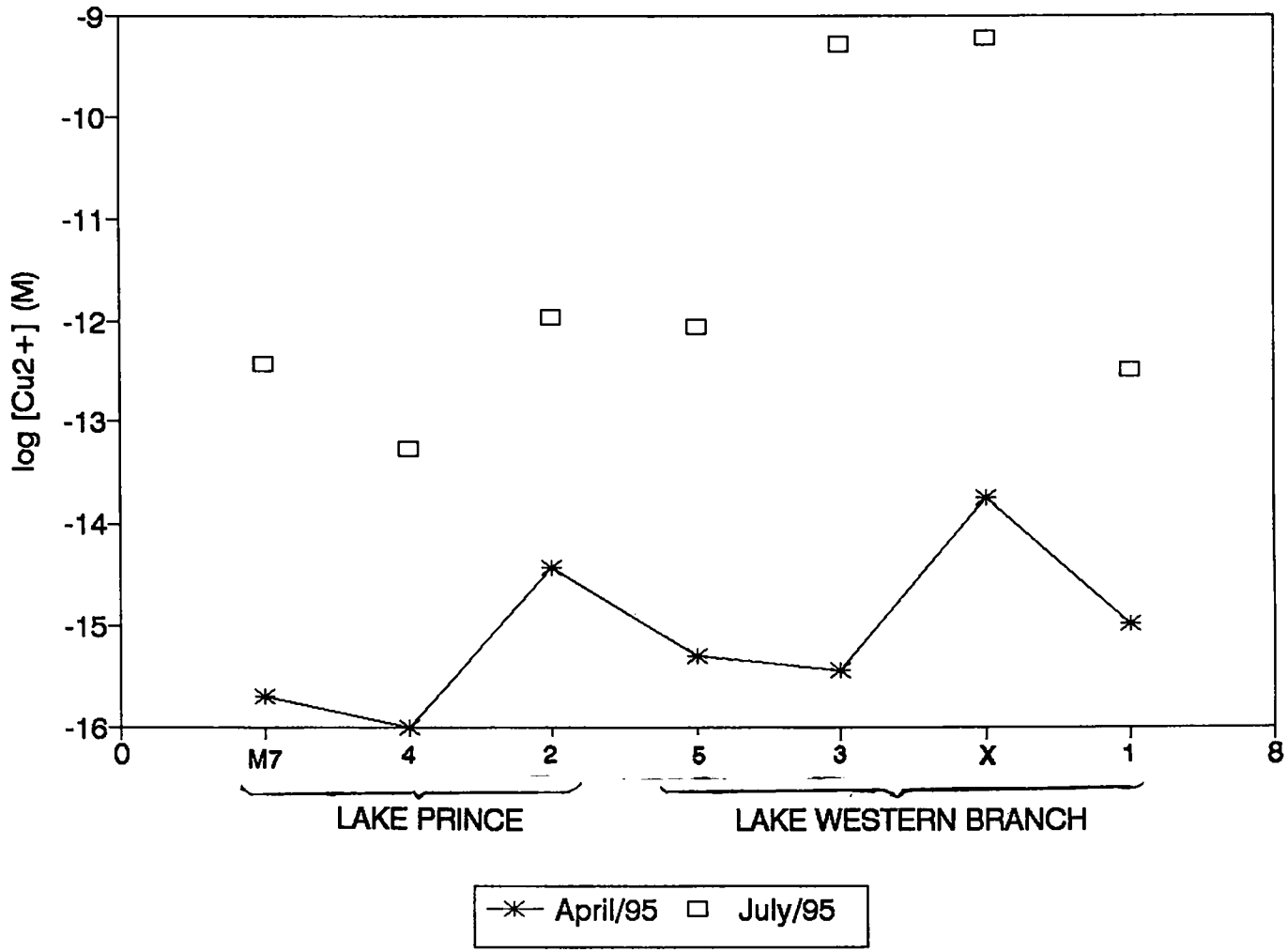
	Stations		
	LP-M7	LP-4	LP-2
C_{L1} (nM)	76.58±0.44	78.74±0.66	55.20±0.67
log K'_{CuL1}	12.80±0.15	13.57±0.34	12.43±0.14
Cu_T (nM)	59.17±0.28	57.94±0.52	44.50±0.53
CuL_1 (nM)	58.41±0.12	57.56±0.06	43.15±0.22
Cu' (pM)	120±30	380±60	120±20
Cu^{2+} (pM)	0.533±0.012	0.074±0.004	1.54±0.022

pM = 10^{-12}


90
80
70
60
50
40
30
20
10
0
[L1] (nM)
LP-M7
LP-4
LP-2
WB-5
WB-3
WB-X
WB-1
April/95
July/95

observations of Xue and Sigg (1993) for Lake Greifen.

In Lake Western Branch, Cu^{2+} concentrations ranged from $1.5x10^{-13}$ to $4.7x10^{-10}$ M (estimated) in September 1994, from $0.35x10^{-14}$ to $9.99x10^{-14}$ M (estimated) in March 1995, from $0.49x10^{-14}$ to $24x10^{-14}$ M (calculated from measurements) in April 1995, and from $4.65x10^{-13}$ to $0.83x10^{-9}$ M (calculated from measurements) in July 1995. In Lake Prince, Cu^{2+} concentrations ranged from $0.97x10^{-14}$ to $93.5x10^{-14}$ M (estimated) in September 1994, from $0.11x10^{-14}$ to $4.03x10^{-14}$ M (estimated) in March 1995, from $0.13x10^{-14}$ to $5x10^{-14}$ M (calculated from measurements) in April 1995, and from $0.74x10^{-13}$ to $15.4x10^{-13}$ M (calculated from measurements) in July 1995. Thus, Cu^{2+} concentrations were highest in both lakes in July (see Table 2 and Figure 12). These levels of Cu^{2+} are comparable to, or higher than those measured for Lake Greifen (10^{-16} to 10^{-14} nM: Xue and Sigg, 1993), and they are in a range that could potentially be toxic to several algal species (Sunda, 1994; Brand et al., 1986). However, organic complexation in Lakes Western Branch and Prince dominates the speciation of total dissolved copper and seems to control Cu^{2+} concentrations; and copper is complexed by strong natural ligands at high concentrations. This situation seems to buffer Cu^{2+} concentrations at their highest levels. Concentrations of free Cu^{2+} ion are believed to control copper toxicity and assimilation by phytoplankton. Sunda (1994) reported that the presence of very strong copper-complexing ligands can protect phytoplankton from copper toxicity by reducing Cu^{2+} concentrations. Brand et al. (1986) observed that several freshwater cyanobacteria and green algae excrete strong ligands with high affinity for copper. Xue and Sigg (1990) measured the copper-complexing strength of a freshwater, green algal exudate and found it to have a value (log K' >13) similar to what they measured for the stronger of two natural copper-complexing ligand classes in


-9
-10
-11
-12
-13
-14
-15
-16
log [Cu2+] (M)
0
M7
4
2
5
3
X
1
8
LAKE PRINCE
LAKE WESTERN BRANCH
April/95
July/95

Lake Greifen (Xue and Sigg, 1993), which they interpreted as suggesting that the copper-complexing ligands in Lake Greifen were derived mainly from algal production (Xue and Sigg, 1993). That both total dissolved copper and L_1 concentrations in Lakes Western Branch and Prince were higher in July than in April (Figure 10) may suggest that L_1 concentrations increase with increasing biological response to higher total copper concentrations. However, despite the relatively high concentrations of strong copper-complexing ligands and the high extent of organic complexation existing in Lakes Western Branch and Prince, the resulting concentrations of Cu^{2+} present in these lakes in July 1995 are high enough (with respect to the toxicity estimates of Brand et al. (1986) and Sunda (1990) to potentially be toxic to the algae in these lakes.

V. CONCLUSIONS

Concentrations of total dissolved copper in Lake Western Branch were 2 to 3 times higher than those in Lake Prince in September, March and April. However, total dissolved copper concentrations in both lakes were similar in July. Total dissolved copper concentrations increased from 2 to 5 times in Lake Western Branch and from 5 to 10 times in Lake Prince, from September to July. These increases may be due to temporal variations in the natural sources and sinks of copper in these lakes, and/or to the intentional additions of copper sulfate to control algal blooms.

LC/DPCSV results indicated that more than 99 % of the total dissolved copper in both Lakes Western Branch and Prince was organically complexed. The concentrations of the strong ligand class, L_1, in both lakes were 2 times higher in July than April. The increases in the L_1 concentrations could be due to increased productivity of the algal communities in these lakes, increased inputs of organic matter from the streams feeding the lakes, and/or to the intentional additions of Cutrin, the copper chelator added along with copper sulfate to control algal blooms. Thus, concentrations of both total dissolved copper and L_1 were higher in both lakes in July compared to April, but the increase in total dissolved copper was greater than for L_1. This differential increase in total dissolved copper relative to L_1 resulted in dramatic increases in the Cu^{2+} concentrations in both lakes. In Lake Western Branch, Cu^{2+} concentrations were 30 to 10^5 times greater in July than in April, while in Lake Prince, Cu^{2+} concentrations were 30 to 200 times greater. Thus, although L_1 concentrations increased in July relative to April, the increase was not sufficient to completely buffer the free Cu^{2+} concentrations from the increase in total dissolved copper. Consequently, the free Cu^{2+} concentrations increased in July relative to April.

The free Cu^{2+} concentrations in these lakes in July are similar in magnitude to those reported to be toxic to algae. However, blooms of cyanobacteria, the species supposed to be most sensitive to Cu^{2+} concentrations, were still present in these lakes in July when Cu^{2+} concentrations were the highest. This may suggest that other factors are influencing the toxicity of Cu^{2+} to the algae in these lakes, and/or that the algae developed a higher tolerance to Cu^{2+}, perhaps due to repeated dosing of the lakes with copper sulfate.

The determination of the degree of complexation of copper has management implications because of the relative importance of chemical and biological uptake processes. Profiles of free Cu^{2+} ion concentrations on a time scale are important for speciation and copper complexation in the upper water column due to impacts of ecological problems and should be included in the future to attempt the control of trace metals in freshwaters.

VI. REFERENCES

Anderson, D.M., Lively, J.S., and Vaccaro, R.F., 1984. Copper complexation during spring phytoplankton blooms in coastal waters. J. Mar. Res., 42: 677-695.

Anderson, M. A. and Morel, F. M. M., 1982. The influence of aqueous iron chemistry on the uptake of iron by the coastal diatom *Thalassiosira wiessflogii*. Limnol. Oceanogr., 27; 789-813.

Apte, S. C., Gardner, M. J., and Ravenscroft, J. E., 1990. An investigation of copper complexation in the Severn estuary using differential pulse cathodic stripping voltammetry. Mar. Chem., 29: 63-75.

Batley, G. E. and Farrar, Y. J., 1978. Irradiation techniques for the release of bound heavy metals in natural waters and blood. Anal. Chim. Acta, 99: 283.

Belli, S. L. and Zirino, A., 1993. Behavior and calibration of the copper (II) ion-selective electrode in high chloride media and marine waters. Anal. Chem, 65: 2583-2589.

Brand, L. E., Sunda, W. G., and Guillard, R. R. L., 1983. Limitation of marine phytoplankton reproductive rates by zinc, manganese, and iron. Limnol. Oceanogr., 28: 1182-1195.

Brand, L. E., Sunda, W. G., and Guillard, R. R. L., 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. J. Exp. Mar. Biol. Ecol., 96, 225.

Borg, H., 1995. Trace elements in lakes. In Salbu, B. and Steinnes, E. (eds),

Trace Elements in Natural Waters. CRC Press, Inc., 177-201.

Bruland, K. W., Franks, R. P., Knauer, G. A. and Martin, J. H., 1979. Sampling and analytical methods for the determination of Cu, Cd, Zn and Ni at the nanogram per liter level in seawater. Anal. Chim. Acta, 99: 275 - 282.

Bruland, K.W. and R. P. Franks, 1983. Mn, Ni, Cu, Zn, and Cd in the Western North Atlantic. In Wong C. S. et al. (eds), Trace Metals in Seawater. Plenum Press, N. Y., 395-414.

Bruland, K. W., Coale, K. H. and Mart, L., 1985. Analysis of seawater for dissolved Cd, Cu, and Pb: an intercomparison of voltammetric and atomic absorption methods. Mar. Chem., 17: 285-300.

Buckley, P. J. M. and van den Berg, C. M. G., 1986. Copper complexation profiles in the Atlantic Ocean. A comparative study using electrochemical and ion exchange techniques. Mar. Chem., 19: 281-296.

Buffle, J., 1988. Complexation reactions in aquatic systems; an analytical approach. Ellis Horwood, Chichester.

Byrne, R. H., Kump, L. R. and Cantrell, K. J., 1988. The influence of temperature and pH on trace metal speciation in seawater. Mar. Chem., 25: 163-181.

Cabaniss, S. E., 1986. Titrator: chemical equilibrium speciation. User's Manual, version 2.1.

Campos, M. L. A. M. and van den Berg, C. M. G., 1994. Determination of copper complexation in seawater by cathodic stripping voltammetry and ligand competition with salicylaldoxime. Anal. Chim. Acta., 284: 481-496.

Coale, K. H. and Bruland, K. M., 1988. Copper complexation in the Northeast Pacific. Limnol. Oceanogr., 33: 1084-1101.

Coale, K. H. and Bruland, K. W., 1990. Spatial and temporal variability in copper complexation in the North Pacific. Deep-Sea Res., 47(2): 317-336.

Davis, J. A. and Leckie, J. O., 1978. Effect of adsorbed complexing ligands on trace metal uptake by hydrous oxides. Environ. Sci. Technol., 12: 1309.

Davis, J. A., 1984. Complexation of trace metals by adsorbed natural organic matter. Geochim. Cosmochim. Acta, 48: 679.

Donat, J. R., Statham, P. J., and Bruland, K. W., 1986. An evaluation of a C-18 solid phase extraction technique for isolating metal-organic complexes from central north Pacific Ocean waters. Mar. Chem., 18: 85-99.

Donat, J. R., and Bruland, K.W., 1992. Organic copper complexation in the Sargasso Sea: comparison to the North Pacific. EOS, Transactions of the American Geophysical Union, 72 (51): 44.

Donat, J. R. and van den berg, C. M. G., 1992. A new cathodic stripping voltammetric method for determining organic copper complexation in seawater. Mar. Chem., 38: 69-90.

Donat, J. R., Lao, K. A. and Bruland, K. W., 1994. Speciation of dissolved copper and nickel in south San Francisco Bay: a multi-method approach. Anal. Chim. Acta, 284: 547-571.

Donat, J. R., 1994. The speciation of copper and cadmium in the Chesapeake Bay. EOS, Transactions American Geophysical Union, 75 (44).

Donat, J. R. and Bruland, K. W., 1995. Trace elements in the oceans. In Salbu, B. and Steinnes, E. (eds), Trace Elements in Natural Waters. CRC Press,

Inc., 247-281.

Duinker, J. C. and Kramer, C. J. M., 1977. An experimental study on the speciation of dissolved zinc, cadmium, lead, and copper in river Rhine and North Sea water by differential pulse anodic stripping voltammetry. Mar. Chem., 5: 207-228.

Flegal, A. R., Smith, G. J., Sanudo-Wilhelmy, S., Gill, G. A. and Anderson, L. C. D., 1991. Dissolved trace element cycles in the San Francisco Bay Estuary. Mar. Chem., 36: 329-363.

Florence, T. M., 1989. Electrochemical techniques for trace element speciation in waters. In Batley, G. E. (eds), Trace Element Speciation: Analytical Methods and Problems. CRC Press, Inc., 77-107.

Hanson, A. K., and Quinn, J. G., 1993. The distribution of dissolved and organically complexed copper and nickel in the middle Atlantic Bight. Can. J. Fish. Aquat. Sci., 20: 151-161.

Hering, J. G., Sunda W. G., Ferguson, R. L., and Morel, F. M. M., 1987. A field comparison of two methods for the determination of copper complexation: bacterial bioassay and fixed-potential amperometry. Mar. Chem., 20: 299.

Huizenga, D. L., and Kester, D. R., 1983. The distribution of total electrochemically available copper in the northwestern Atlantic Ocean. Mar. Chem., 13: 281-292.

MacKey, D., 1982. Cation-exchange behavior of a range of absorbants and chromatographic supports with regard to their suitability for investigating trace metal speciation in natural waters. J. Chromatogr., 242: 275-287.

McKnight, D. M. and Morel, F. M. M., 1979. Release of weak and strong copper complexing agents by algae. Limnol. Oceanogr., 24 (56): 823-837.

Martin, J. H., Bruland, W. K., and Broenkow, W. W., 1976. Cadmium transport in the California current. In Windom, H. L., and Duce R. A. (eds), Marine Pollutant Transfer. D. C. Heath, Lexington MA, 159-184.

Mills, G. L., and Qinn, J. G., 1981. Isolation of dissolved organic matter and copper-organic complexes from estuarine waters using reverse-phase liquid chromatography. Mar. Chem., 10: 93-102.

Moffett, J. W. and Zika, R. Z., 1987. Solvent extraction of copper acetylacetonate in studies of copper (II) speciation in seawater. Mar. Chem., 21: 301.

Moffett, J. W., Brand, L. E., and Zika, R. G, 1990. Distribution and potential sources and sinks of copper chelators in the Sargasso Sea. Deep-Sea Res., 37: 27-36.

Moffett, J. W., 1995. Temporal and spatial variability of copper complexation by strong chelators in the Saragasso Sea. Deep-Sea Research I, 42 (8): 1273-1295.

Morel, F. M. M., and Hering, J. G., 1993. Principles and applications of aquatic chemistry. Wiley-Interscience, New York.

Morrison, G. M. P., 1989. Trace element speciation and its relationship to bioavailability and toxicity in natural waters. In Batley, G. E. (ed), Trace Element Speciation: Analytical Methods and Problems. CRC Press, Inc., 25-40.

Peterson, R., 1982. Influence of copper and zinc on the growth of a freshwater

alga, *Scendesmus quadricauda*: the significance of chemical speciation. Environ. Sci. Technol., 16: 443.

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.

Salbu, B., and Steinnes, E., 1995. State of the art and future trends. In Salbu, B. and Steinnes, E. (eds),Trace Elements in Natural Waters. CRC Press, Inc., 283-287.

Salomons, W. and Baccini, P., 1986. Chemical species and metal transport in lakes. In Bernhard, M., Brinckman, F. E., and Sadler, P. J. (eds), The Importance of Chemical Speciation in Environmental Processes, Springer-Verlag, Berlin, 193-216.

Schafran, G. C. and Scully, F. E., 1993. Water quality monitoring of Lakes Prince and Western Branch: 1993. ODU Research Foundation. Technical memorandum no. 11 B. phase- pilot testing, 2-23.

Sigg, L., 1985. Metal transfer mechanisms in lakes; the role of settling particles. In Stumm, W., Chemical processes in lakes. John Wiley & Sons, Inc., 283-310.

Sigg, L. and Xue, H., 1994. Metal speciation; concepts, analysis and effects. In Bidoglio, G. and Stumm, W. (eds), Chemistry of Aquatic Systems: Local and Global Perspectives, 153-181.

Stoecker, D. K., Sunda, W. G., and Davis, L. H., 1986. Effects of copper and zinc on two planktonic ciliates. Mar. Biol., 92, 21.

Sunda, W. G. and Guillard, R. R. L., 1976. The relationship between cupric ion

activity and the toxicity of copper to phytoplankton. J. Mar. Res., 34: 511-529.

Sunda, W. G., Engel, D. W. and Thuotte, R. M., 1978. Effect of chemical speciation on the toxicity of cadmium to grass shrimp: importance of the free cadmium ion. Environ. Sci. Technol., 12: 409-413.

Sunda, W. G. and Ferguson, R. L., 1983. Sensitivity of natural bacterial communities to additions of copper and to cupric ion activity: a bioassay of copper complexation in seawater. In Wong, C. S., Boyle, E., Bruland, K. W., Burton, J. D. and Goldberg, E. D. (eds), Trace Metals in Sewater. Plenum Press, New York, 871-891.

Sunda, W. G., 1984. Measurement of manganese, zinc and cadmium complexation in seawater using chelex ion exchange equilibria. Mar. Chem., 14: 365-378.

Sunda, W. G. and A. K. Hanson, 1987. Measurement of free cupric ion concentration in seawater by a ligand competition technique involving copper sorption onto C_{18} Sep-Pak cartridges. Limnol. Oceanogr., 32: 537-551.

Sunda, W. G., 1990. Trace metal interactions with marine phytoplankton. Biol. Oceanogr., 6; 411-442.

Sunda, W. G., Tester, P. A. and Huntsman, S. A., 1990. Toxicity of trace metals to *Arcatia tonsa* in the Elizabeth River and Southern Chesapeake Bay. Estuarine, Coastal and Shelf Science, 30: 207-221.

Sunda, W. G. and Huntsman, S.A., 1991. The use of chemiluminescence and ligand competition with EDTA to measure copper concentration and

speciation in seawater. Mar. Chem., 36: 137-163.

Sunda, W. G., 1994. Trace metal/ phytoplankton interactions in the sea. In Bidoglio, G. and Stumm, W. (eds), Chemistry of Aquatic Systems: Local and Global Perspectives. 213-247.

Turner, D. R., Whitfield, M. and Dickson, A. G., 1981. The equilibrium speciation of dissolved components in freshwater and seawater at 25°C and 1 atm. pressure. Geochim. Cosmochim. Acta, 45: 855-881.

van den Berg, C. G. M., and Kramer, J. R., 1979. Determination of complexing capacities of ligands in natural waters and conditional stability constants of the copper complexes by means of manganese dioxide. Anal. Chim. Acta, 106: 113-120.

van den Berg, C. G. M., 1982. Determination of copper complexation with natural organic ligands in seawater by equilibration with MnO_2. Experimental procedures and application to surface water. Mar. Chem., 11: 323-342.

van den Berg, C. M. G., 1984. Determination of the complexing capacity and conditional stability constants of complexes of copper (II) with natural organic ligands in seawater by CSV of copper-catechol complex ions. Mar. Chem., 15: 1-18.

van den Berg, C. G. M. and Dharmvanij, S., 1984. Determination of complexing capacities of zinc using equilibration of MnO_2 with zinc. Limnol. Oceanogr., 29: 1025-1036.

van den Berg, C. M. G., 1986. Determination of copper, cadmium and lead in seawater by cathodic stripping voltammetry of complexes with 8-

hydroxyquinoline. J. Electroanal. Chem., 215: 111-121.

van den Berg, C. M. G., Nimmo, M., Daly, P. and Turner, D. R., 1990. Effects of the detection window on the determination of organic copper speciation in estuarine waters. Anal. Chim. Acta, 232; 149-159.

van den Berg, C. M. G. and Donat, J. R., 1992. Determination and data evaluation of copper complexation by organic ligands in seawater using cathodic stripping voltammetry at varying detection windows. Anal. Chim. Acta, 257: 281-291.

Waite, T. D. and Morel, F. M. M., 1983. Characterization of complexing agents in natural waters by copper(II)/ copper(I) amperometry. Anal. Chem., 55: 1268-1274.

Xue, H. B., and Sigg, L., 1990. Binding of Cu(II) to algae in a metal buffer. Water Res., 24(9): 1129-1136.

Xue, H. and Sigg, L., 1993. Free cupric ion concentration and Cu(II) speciation in a eutrophic lake. Limnol. Oceanogr., 38 (6): 1200-1213.

Zevenhuisen, L. P. T. M., Dolfing, J., Eshuis, E. J., and Scholten-Koersehman, I. J., 1979. Inhibitory effects of copper on bacteria related to the free ion concentration. Microbiol. Ecol., 5: 139.