Strong copper complexation in an organic-rich estuary: the importance of allochthonous dissolved organic matter

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Abstract

River input of allochthonous organic matter dominates the strong complexation capacity of dissolved copper in the organic-rich Cape Fear River (CFR) estuary, North Carolina. This slightly stratified estuary is characterized by conservatively mixed dissolved organic carbon (DOC = 200–1200 μM C), high river flow, and low biological productivity. Copper speciation data measured using competitive ligand equilibration–cathodic stripping voltammetry (CLE–CSV with 8-hydroxyquinoline (8-HQ)) for seven Cape Fear estuarine transects revealed that strong (mean detection window [Cu8HQ = 105.2]) Cu-complexing ligands range in concentration from 7 to >200 nM (at fixed $K_{CuL} = 10^{13.5}$), are conservatively mixed below the turbidity maximum zone ($S_f < 5$), and exist in substantial excess of dissolved Cu levels (3–25 nM). Strong ligand and DOC concentrations exhibited strong linear correlations among transect samples at DOC concentrations < 1000 μM C ($r^2 = 0.93$, $p < 0.01$) and among all size fractions of ultrafiltered estuarine samples ($r^2 = 0.94$, $p < 0.01$). A 300 μM C solution of Cape Fear River humic substances isolated using C_{18} extraction exhibited a strong Cu ligand concentration of 143 nM (at fixed $K_{CuL} = 10^{13.5}$). Comparison with speciation data from estuarine transects indicates C_{18} isolated humics could account for 100% of the typical strong Cu-complexation capacity in the Cape Fear estuary.

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1. Introduction

The speciation of dissolved copper controls its bioavailability as well as its geochemical cycling. In coastal waters, total dissolved Cu concentrations (TDCu) typically range from 3 to 50 nM, whereas free Cu^{2+} levels are usually $< 1$ nM as a consequence of organic complexation (Donat et al., 1994; Kozelka and Bruland, 1998). Most current research indicates ligands with strong binding ability (i.e., conditional stability constant of Cu-ligand complex $> 10^{11}$) control the bioavailability of Cu^{2+} ions in marine waters (Donat and Bruland, 1995). Weaker binding complexants regulate speciation only as TDCu levels increase, typically in areas of high anthropogenic influence (Donat et al., 1994; Moffett et al., 1997; Kogut and Voelker, 2001). Complexation by strong binding ligands may promote
dissolved Cu transport in marine (Van den Berg et al., 1987; Apte et al., 1990) and freshwater systems (Breault et al., 1996) by inhibiting its adsorption to particles. The flux of Cu from an estuary to the coastal ocean is likely enhanced for estuarine systems with low residence times as the transfer of Cu from strongly binding dissolved moieties to particles can be kinetically hindered (Achterberg et al., 2002).

Chemical structures of strong Cu-binding ligands in aquatic systems remain unresolved, but researchers have elucidated many of their sources. In open ocean and productive coastal waters, autochthonous biologically produced ligands may dominate the complexing capacity of dissolved Cu. Correlations between primary productivity and strong ligand distribution have been observed in marine systems (Moffett et al., 1990) as well as in productive freshwaters (Xue and Sigg, 1999). A host of organisms have been identified as producers of extracellular strong Cu-complexing ligands including cyanobacteria (Moffett and Brand, 1996), dinoflagellates (Croot et al., 2000), heterotrophic bacteria (Gordon et al., 2000), and coccolithophores (Leal et al., 1999). It has been proposed that high affinity Cu ligands can be exuded as a feedback mechanism against the potential toxicity of Cu$^{2+}$ ions (Moffett and Brand, 1996; Gordon et al., 2000).

Some reports suggest terrestrially derived ligands might exhibit weaker binding capabilities than those produced in situ biologically (Moffett et al., 1997; Muller et al., 2001). However, in estuarine systems where the dissolved organic matter (DOM) pool is dominated by a river flux of terrestrially derived compounds, it is likely some portion of this bulk material exhibits a high affinity for Cu (Xue and Sigg, 1999), although the functional groups responsible for binding have not been clearly identified. Rozan and Benoit (1999) reported a strong relationship between DOM and Cu-binding capacity in four New England rivers. Copper speciation analyses performed on fulvic and humic acid solutions made from Suwannee River standards (SRFA and SRHA—International Humic Substance Society standards) revealed that humic substances exhibit strong binding characteristics at concentrations typical of marine waters (Xue and Sigg, 1999; Kogut and Voelker, 2001). Computer-based binding models have also predicted that humic substances possess strong Cu ligand characteristics (Hamilton-Taylor et al., 2002). However, few studies have measured the role of river input as a major source of strong ligands to estuaries and coastal waters (Apte et al., 1990; Gerringa et al., 1996), and no field studies have directly determined the contribution of humics to the strong Cu-complexation capacity within a specific estuary.

This paper addresses the role of allochthonous DOM as the primary source of strong Cu ligands to the river-dominated Cape Fear estuary in southeastern North Carolina. This is the first detailed Cu speciation study conducted in one of the organic-rich estuaries of the southeastern U.S. Additionally, this study is the first to compare detailed Cu speciation characteristics of an estuary with the strong Cu-complexation capacity of humic substances extracted from the same estuary.

2. Methods

2.1. Study location

The Cape Fear estuary is located between the cities of Wilmington and Southport along the southeastern coast of North Carolina (Fig. 1). This system has a deep river channel (~15 m) and is characterized by voluminous inputs of organic substances from upstream freshwater swamps and two blackwater tributaries (Black River and Northeast Cape Fear River). Dissolved organic carbon (DOC) concentrations are very high ranging from 200 to 1200 µM C (Avery et al., 2003). Typical humic concentrations at the freshwater endmember are 30–40 mg l$^{-1}$ (R. Kieber, unpublished data). Semi-diurnal tides of ~1.5 m combined with high river flow produce short estuarine residence times (<1 week). Primary productivity is very low due to light limitation and short residence times (Mallin et al., 1999a). The Cape Fear is an ideal estuary for examining river inputs of strong Cu-binding ligands because of the relatively short distance over which the major estuarine processes interact (60 km).

2.2. Estuarine transects

Sampling locations were chosen so that speciation characteristics could be analyzed over the maximum salinity range within the Cape Fear estuary (Fig. 1).
Three sites (M18, M35, M54 or M61) located below the confluence of the Cape Fear River (CFR) and this estuary’s largest tributary, the Northeast Cape Fear River (NECFR), were sampled from a boat during 11 transects from July 1998 to September 2000. Samples were also collected during seven of these transects upstream of this confluence at one site in both the CFR (NAV station) and NECFR (NCF6 station). Water depths at each sampling station are >13 m with an average cross-estuary depth of 6–8 m (detailed description of each station in Mallin et al., 1999b). Samples were collected as close to high tide as possible to attain the maximum salinity gradient. However, because of large variations in river flow, there is considerable variability in the salinity regime at each station (Mallin et al., 1999b).

All sampling was conducted using trace metal clean techniques. All bottles, sample tubing, and filters were cleaned with a series of weeklong acid soakings including 2 M HNO₃ and 3 M HCl, followed by storage in MQ water (Millipore Milli-Q Plus system; 18.2 MΩ cm⁻¹) brought to pH ~ 2 with trace metal grade HCl (Fisher). Bottles and tubing were stored in dust-free plastic bags until use.

Copper speciation, TDCu, and dissolved organic carbon samples were collected at a depth of 1.0–1.5 m using a peristaltic pump connected to Teflon®-lined Tygon® tubing extended approximately 3 m away and upstream from the boat with a PVC pole. Sample water was filtered upon collection using trace metal clean in-line cartridge filters (Meissner), beginning with a 1.2 µm polypropylene filter followed by a 0.2 µm polyethersulfone filter (nominal “dissolved” filter sizes). Speciation (FLPE—fluorinated polyethylene) and TDCu (HDPE—high density polyethylene) bottles were placed within two separate clean plastic bags to prevent contamination. Speciation samples were placed on ice until return to the laboratory, where they were stored frozen at −20 °C. Duplicate speciation analyses on frozen and non-frozen samples revealed no detectable storage artifacts. Total dissolved Cu samples were acidified to pH ~ 2 using ultrapure HNO₃ (Fisher Optima). Filtered DOC samples were stored in the dark at 2–4 °C until analysis, usually within 2–3 days. Salinity and water temperature data were collected in situ using a YSI 85 temperature/conductivity probe. Sample pH values were measured immediately upon return to the laboratory with a Corning pH electrode calibrated with NBS buffers.

2.3. Cu speciation analysis

Copper speciation analyses were conducted using competitive ligand equilibration–cathodic stripping voltammetry (CLE–CSV) with 8-hydroxyquinoline (8-HQ) as the competing ligand (Donat et al., 1994). A detection window (ΔCu₀₈-HQ ~ 10⁵) was chosen to target the examination of strong Cu-complexing
ligands. The possibility that large concentrations of weaker ligands would also be detected using these experimental conditions will be discussed later. The detection window is a function of the conditional stability constant and concentration of the 8-HQ at a specified ionic strength and pH (Donat et al., 1994). Calculation of the competition level \((\frac{[Cu^{2+}]}{[Cu^{2+}]/[\Sigma L_i]} + 1/Ke_{CuL}[\Sigma L_i])\). 

Ligand concentrations \((\Sigma L_i)\) were calculated with a fixed \(Ke_{CuL}\) value of \(10^{13.5}\) in accordance with the approximate midpoint of \(pCu\) values (12–15) determined from voltammetric results (Voelker and Kogut, 2001). Values for \([Cu^{2+}]\) and \([CuL]\) used in determining \(\Sigma L_i\) with 8-HQ as the competing ligand were calculated as detailed in Donat et al. (1994). Issues associated with fitting titration data using a one-ligand model are discussed later in this section.

2.4. TDCu analysis

Previously acidified samples (pH ~ 2) were UV-irradiated with a 1.2 kW high pressure Hg-vapor lamp for 4–6 h prior to TDCu analysis to release Cu from all binding complexes. Total dissolved Cu concentrations were determined by CSV based on a procedure in Van den Berg (1986). Briefly, 7–10 ml of sample were brought to a pH of 7.7–8.2 using ultrapure 5 M NH4OH (Fisher Optima), 70–100 \(\mu\)l of 1 M HEPES buffer were added (0.01 M), followed by 70–100 \(\mu\)l addition of 1000 \(\mu\)M 8-HQ (10 \(\mu\)M). Samples were analyzed in triplicate using the method of standard additions with a typical precision of \(\pm 1.0–1.5\) nM. Square wave scan parameters included a \(-1.1\) V deposition potential, 10–20 s deposition time, 2 mV step, 25 mV pulse height, scan rate of 100 mV s\(^{-1}\) (50Hz), and a scan range of \(-0.2\) to \(-0.65\) V. Accuracy was verified frequently by analyzing known analytical standards (NRC Canada NASS-5, CASS-3, SLEW-3, and SLRS-4); values obtained were always within certified 95% confidence levels.

2.5. DOC analysis

Dissolved organic carbon concentrations were analyzed in triplicate with a high temperature catalytic oxidation method using a Shimadzu TOC 5000 analyzer as described in Avery et al. (2003). Samples
were diluted with MQ water when necessary to fit within the standard curve produced for each analysis. Standard deviation for triplicate injections were <2% and the detection limit was approximately 5 μM. Accuracy of the DOC analysis was verified by analyzing ampoules of BATS deep ocean certified reference material (CRM) intermixed within sample analyses. A value of 48.1 ± 4.3 μM C \( (n=18) \) was obtained for the CRM, comparable to the published value of 47.4 ± 3.1 \( (n=14) \) presented in Table 6 of Sharp et al. (2002).

### 2.6. Ultrafiltration procedure

Samples were collected for ultrafiltration analyses using trace metal clean procedures near M18 and M35 (see Fig. 1) in April 2002. Feed samples were filtered on-site using 0.45 μm filters (Meissner) and collected in 12.5 L FLPE carboys. The ultrafiltration technique (ultrafiltration conducted at UNCW by M. Shafer’s research group from the University of Wisconsin at Madison) employed a cross-flow filtration system using a Teflon® diaphragm pump (KNF-Neuberger) operating at a cross-flow rate of 3–5 l/min. Millipore Prep-Scale TFF spiral-wound ultrafiltration cartridges (polysulfone) were used with 1000 Da (1 kDa) and 10,000 Da (10 kDa) membrane filters made of regenerated cellulose. Two samples were produced for each ultrafiltration, a filter passing fraction (permeate) and a non-filter passing fraction (retentate). The efficiency of the ultrafiltration procedure was verified by mass balance calculations using DOC concentrations in the feed, permeate, and retentate samples with appropriate volume concentration factors (recovery ranged from 99% to 115%). Strong Cu ligand and TDCu concentrations were measured directly from the following fractions: <0.45 μm (feed), 10 kDa permeate, and 1 kDa permeate. The smallest fraction (1 kDa permeate) is operationally defined as ‘truly dissolved’ (Muller, 1996).

### 2.7. Preparation of Cape Fear humic solutions

Humic substances were isolated using Sep Pak® C\textsubscript{18} columns from a Cape Fear River water sample collected near station M61 (see Fig. 1 for location) during a separate study in 1996 (Kieber et al., 1999). The water sample had been collected at low salinity (~2) and contained an ambient humic concentration of ~35 mg l\(^{-1}\) (R. Kieber, unpublished data). Following passage of the water sample through the C\textsubscript{18} column, MQ water was passed through the column to remove all salts, followed by methanol elution of the humics. Extracted material was separated from the methanol by evaporating the solvent using vacuum drying procedures. The dried humic had been stored in the dark at 4 °C in a sealed 20 ml Teflon® vial. Potential artifacts in speciation measurements associated with the isolation and storage of collected humic substances could not be determined since the original water sample from which the humics had been extracted had been previously discarded.

To evaluate the strong Cu-complexation capacity of the humic extract, a stock humic solution was prepared by dissolving the C\textsubscript{18} extracted material in MQ water. The stock solution consisted of 26 mg extract dissolved in a 1 L solution with a salinity of ~5. From this stock solution, secondary humic solutions were prepared to mimic typical salinity and humic concentrations observed in the Cape Fear estuary by diluting with the appropriate volume of MQ water and UV-irradiated seawater (previously collected near Gulf Stream waters ~100 km off the coast of North Carolina). All humic solutions were analyzed for DOC, ΣL\textscript{i}, and TDCu concentrations as described previously.

### 2.8. Terminology and data analysis

Organic metal-complexing ligands have typically been divided into strong (L\textsubscript{1} class—log \( K_{Cu}^{L_{1}} \geq 11 \)) and weaker (L\textsubscript{2}, L\textsubscript{3} classes—log \( K_{Cu}^{L_{2,3}} \approx 8–11 \)) ligand classes based on their conditional stability constants. Although these class distinctions have been used extensively, the pervasive inconsistencies of this ‘ligand class’ terminology have been noted (Town and Filella, 2000). Voelker and Kogut (2001) argued that distinctions between L\textsubscript{1} and L\textsubscript{2} are arbitrary, especially in waters where mixtures of ligands exist. Another central argument in metal speciation studies has centered on whether speciation data should be fitted to discrete ligand models where metals are assumed to occupy specific binding sites within a substance or to continuous distribution models where metal attachments are more random and include electrostatic
forces (Dzombak et al., 1986; Buffle et al., 1990). Because of the caveats associated with speciation evaluations, L₁ terminology is not used in this paper.

For this study, titration data were evaluated using a one-ligand model, but this is not intended to imply that only one strong Cu ligand exists within the Cape Fear system. On the contrary, it is likely that a spectrum of binding sites (Hudson et al., 2003) are responsible for the Cu²⁺ buffering capacity in the Cape Fear system as reported for other natural waters (Kogut and Voelker, 2001, 2003). Many authors have attempted to model the results of voltammetric analyses using multi-ligand models to better fit the titration data (Cabaniss and Shuman, 1988; Hering and Morel, 1988; Kogut and Voelker, 2001; Voelker and Kogut, 2001; Hudson et al., 2003). In some cases, the titration data are fitted more precisely using multi-ligand models than single ligand models (Voelker and Kogut, 2001). By using a one-ligand model consistently in this study, the presented data represent the sum of ligands of various strengths within the experimental detection window denoted by the term \( \Sigma L_i \). Using a one-ligand model precludes a precise investigation of the fate of distinct Cu complexants, but in an organic-rich system such as the Cape Fear, attempting to discern individual types of binding sites is a very difficult objective. Additionally, data presented here do not specifically address the mechanism by which ambient Cu is bound in the Cape Fear water column. However, our results do allow for a quantification of the Cu-complexation capacity at the aforementioned detection window consistent with other Cape Fear studies (Shank et al., 2004a,b).

A primary goal of speciation measurements is to predict the free Cu²⁺ buffering capacity of a sample as Cu loading increases, and this can be accomplished by examining plots of free Cu²⁺ concentration as a function of ambient Cu concentration available for binding by natural ligands, typically denoted as CuT* (Moffett et al., 1997; Xue and Sigg, 1999; Kogut and Voelker, 2001, 2003; Voelker and Kogut, 2001). Plots of Cu²⁺ vs. CuT*, as well as the non-linear algorithm used in our one-ligand model, require that the sensitivity (i.e., slope of reduction current response to total Cu = nA/nM) of the sample be accurately measured, introducing a well-documented potential caveat in producing accurate speciation data: the ability of the analyst to determine whether all ligands within the particular detection window have been titrated with Cu and whether the reduction current has truly become a linear function of Cu added (Miller and Bruland, 1997; Kogut and Voelker, 2001, 2003; Voelker and Kogut, 2001; Hudson et al., 2003). The sensitivity of a sample can be calculated using internal calibrations, external calibrations, or overload titrations (see Kogut and Voelker, 2001, 2003). If used incorrectly, either by not titrating with enough Cu to produce true linear response or because of large surfactant effects, internal calibrations can substantially underestimate the sensitivity producing underestimated ligand concentrations and lower Cu²⁺ buffering capacities (Kogut and Voelker, 2001; Voelker and Kogut, 2001). The potential for the substantial underestimation of ligand concentrations in this study were minimized by keeping deposition times short to limit surfactant effects (typically ≤ 15 s) and using a high detection window (mean \( z_{CuH}_{150} = 10^{5.2} \)). Kogut and Voelker (2003) reported that internal calibration using a detection window of ~ 10² (using salicylaldoxime as competing ligand) produced a sensitivity value within 4% of the sensitivity value measured using overload titrations for samples collected from Eel Pond near Cape Cod, MA. Given the typical reproducibility in our speciation analyses (typically 10–15%), an error of 4% would be undetectable. When warranted, this paper will provide details of inconsistencies within speciation results and the likelihood that incorrect internal calibration errors were responsible. Additionally, Kogut and Voelker (2003) reported that ambient dissolved Cu may exist in a non-exchangeable form causing an error in the proper calculation of ligand concentrations. As the Cape Fear data will show, however, TDCu levels are small enough and \( \Sigma L_i \) concentrations large enough so that overestimating the exchangeable Cu concentration would have a small effect on calculated \( \Sigma L_i \) concentrations.

3. Results

3.1. Titration plots

Titration plots presented in Figs. 2–4 represent typical CSV results for the Cape Fear samples. Sensitivity measurements were typically reproducible to within 10% for replicate samples producing comparable ligand concentrations (\( \Sigma L_i \)). However, the data in Figs. 2–4 also show that when using internal calibra-
tion to determine sensitivity, the number of points considered within the linear range was not always consistent on duplicate samples. Determining the linear region requires subjective reasoning on the part of the analyst and this is certainly a drawback to any CSV analysis. Determining the sensitivity of CSV analyses on samples collected from the organic-rich waters of the upper Cape Fear estuary (Figs. 5 and 6—NAV and

Fig. 2. Representative results of duplicate CSV analyses for Cape Fear estuarine samples. Open diamonds (○) indicate data points not used in determining the sensitivity (S) of each sample.

Fig. 3. Representative results of duplicate CSV analyses for Cape Fear estuarine samples. Open diamonds (○) indicate data points not used in determining the sensitivity (S) of each sample.
NCF stations) proved more problematic than the lower sites (i.e., M54/61, M35, M18). Substantial underestimation of sensitivity, due either to surfactant effects or incomplete Cu titration of the natural ligands (Kogut and Voelker, 2001, 2003; Voelker and Kogut, 2001), implies that the measured ligand concentrations in our

![Graphs showing CSV analyses results for Cape Fear estuarine samples.](image-url)

Fig. 4. Representative results of duplicate CSV analyses for Cape Fear estuarine samples. Open diamonds (◇) indicate data points not used in determining the sensitivity (S) of each sample.

![Graphs showing CSV analyses results for Cape Fear estuarine samples.](image-url)

Fig. 5. Representative results of duplicate CSV analyses for Cape Fear estuarine samples. Open diamonds (◇) indicate data points not used in determining the sensitivity (S) of each sample.
samples could be underestimated. For the upper estuarine sites (Figs. 5 and 6), titrations were conducted to ~250 nM total Cu with apparent linearity only being observed in the last 2–3 data points. It is possible titration plots would have exhibited curvature past 250 nM; however, higher additions of Cu were not possible because of saturation of the HMDE. Data presented in Figs. 2–4 do not appear affected by underestimated sensitivities since linear responses appeared over a range of Cu concentrations approaching 100 nM.

### 3.2. Estuarine distributions of strong Cu ligands

The speciation of dissolved Cu was determined along transects of the Cape Fear estuary between July 1998 and September 2000. Detection windows (Table 1) were approximately equivalent for all samples (log $\alpha_{Cu8HQ} = 5.2 \pm 0.3$) allowing for a direct comparison of ligand concentrations at varying salinities (Van den Berg et al., 1990; Van den Berg and Donat, 1992). Speciation data are presented (Table 1) for estuarine transects where measurable salinities were observed at all three lower stations M18, M35, and M54/M61 at the time of sampling. Data collected for upstream sites in the Cape Fear (NAV) and Northeast Cape Fear (NCF6) rivers are listed only for sampling trips where salinities measured at least 2 and Cu speciation analyses were performed. Freshwater and low salinity ($S < 2$) samples were not examined because of difficulties in the CSV analyses related to low ionic strength, high surfactant levels, and limitations of the HMDE to produce a linear response to Cu$^{2+}$ added at very high Cu concentrations. Unfortunately, this limitation precludes our data from examining ligand characteristics and variability in the early estuarine mixing zone where rapid changes in dissolved organic material are likely to occur (Sholkovitz et al., 1978; Gustaffson et al., 2000).

Strong Cu ligand concentrations ($\Sigma L_i$ at fixed $K'_{CuL} = 10^{13.5}$) in all samples from the Cape Fear estuary ranged from 7 to 229 nM (Table 1). Strong Cu ligand concentrations in the Cape Fear are comparable to those measured at similar salinities in the Severn Estuary (Apte et al., 1990) and Galveston Bay (Tang et al., 2001), but much higher than reported for Narragansett Bay (Kozelka and Bruland, 1998) and harbors near Cape Cod (Moffett et al., 1997). Additionally, the strong Cu-complexation capacity measured in high salinity waters of the Cape Fear estuary (i.e., site M18) is much higher than typically observed in oceanic waters (Donat and Bruland, 1995). High river flow coupled with ambient high estuarine ligand

Fig. 6. Representative results of duplicate CSV analyses for Cape Fear estuarine samples. Open diamonds (○) indicate data points not used in determining the sensitivity (S) of each sample.
concentrations in the Cape Fear indicates this estuary exports strong Cu-complexing ligands to coastal ocean waters (Shank et al., 2004a).

Estuarine distributions of strong Cu ligands are plotted in Fig. 7a for completed Cape Fear transects. River flow varied by 10-fold during our transects, ranging from 32 m$^3$ s$^{-1}$ in June 1999 to 361 m$^3$ s$^{-1}$ in December 1999 (USGS data at http://waterdata.usgs.gov/nc/nwis), but ligand concentrations appear conservatively mixed under all flow conditions. By combining all transects, a theoretical mixing line was constructed producing an average freshwater endmember strong ligand concentration of 213 nM and a seawater endmember of 3 nM ($\Sigma L_i = 35$), similar to that found (1–2 nM) in Sargasso Sea waters (Moffett, 1995). A conservative strong Cu ligand distribution was also observed during a single transect in the Severn Estuary in the U.K. (Apte et al., 1990). A plot of strong Cu ligand concentration vs. salinity constructed from tabulated data presented in that study yields a mixing curve ($y = -4.8x + 180$, $r^2 = 0.93$, $p < 0.01$) similar to the theoretical curve presented in Fig. 7a. However, the detection window reported by Apte et al. (1990) was log $x \sim 3.4$, significantly lower than used in this study (log $x \sim 5$). Since measured ligand concentrations generally decrease as the detection window increases (Van den Berg et al., 1990; Van den Berg and Donat, 1992), it is possible that the similarities would be less remarkable had the $x$ values been equivalent. Conservative distributions of strong Cu ligands have also been reported in middle to high salinity sections of Galveston Bay (Tang et al., 2001) and the Scheldt estuary (Van den Berg et al., 1987), although no statistical data were presented in either of those studies.

While speciation data presented in Table 1 and Fig. 7a were calculated with a detection window ($x_{Cu8HQ}$)

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<th>Site</th>
<th>Temperature (°C)</th>
<th>Salinity</th>
<th>pH</th>
<th>DOC (μM C)</th>
<th>TDCu (nM)</th>
<th>Detection window log $x_{Cu8HQ}$</th>
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<td>1153</td>
<td>15.6 ± 1.0</td>
<td>5.3</td>
</tr>
<tr>
<td>17-Dec-99</td>
<td>M18</td>
<td>14.0</td>
<td>27.0</td>
<td>7.9</td>
<td>352</td>
<td>6.0 ± 0.5</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>M35</td>
<td>13.5</td>
<td>15.0</td>
<td>7.6</td>
<td>648</td>
<td>10.2 ± 0.7</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>M61</td>
<td>12.3</td>
<td>3.0</td>
<td>7.4</td>
<td>976</td>
<td>12.0 ± 0.5</td>
<td>5.7</td>
</tr>
<tr>
<td>30-Jun-00</td>
<td>M18</td>
<td>28.3</td>
<td>32.0</td>
<td>8.0</td>
<td>242</td>
<td>3.0 ± 0.4</td>
<td>5.0</td>
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<tr>
<td></td>
<td>M35</td>
<td>27.8</td>
<td>25.7</td>
<td>8.0</td>
<td>526</td>
<td>6.2 ± 1.2</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>M61</td>
<td>28.3</td>
<td>8.0</td>
<td>7.6</td>
<td>782</td>
<td>18.3 ± 1.8</td>
<td>5.5</td>
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<tr>
<td></td>
<td>NAV</td>
<td>28.4</td>
<td>2.2</td>
<td>7.4</td>
<td>1336</td>
<td>22.7 ± 1.5</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>NCF6</td>
<td>28.9</td>
<td>3.0</td>
<td>7.3</td>
<td>1242</td>
<td>14.7 ± 2.0</td>
<td>5.4</td>
</tr>
<tr>
<td>20-Sep-00</td>
<td>M18</td>
<td>25.5</td>
<td>31.0</td>
<td>7.9</td>
<td>278</td>
<td>3.8 ± 1.4</td>
<td>5.0</td>
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<tr>
<td></td>
<td>M35</td>
<td>25.3</td>
<td>14.7</td>
<td>7.8</td>
<td>724</td>
<td>10.7 ± 0.7</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>M61</td>
<td>25.2</td>
<td>3.3</td>
<td>7.4</td>
<td>1286</td>
<td>14.2 ± 1.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>

TDCu = mean ± S.D., n = 3; $\Sigma L_i$ = mean ± 0.5 × range at fixed $K_{CuL}^V = 10^{13.5}$, n = 2.
of $\sim 10^5$ and are denoted as “strong” ligands, it must be mentioned that some of the ligands detected could be weaker ligands existing at very high concentrations. Kogut and Voelker (2003) modeled a sample from Waquoit Bay as having approximately 300 nM of weaker ligand with a conditional stability constant of $10^{10}$. Given that the DOC concentration of the Waquoit Bay sample was 2.3 mg l$^{-1}$, approximately 20% of the DOC concentration in the upper estuarine waters of the Cape Fear, if the binding capability of the organic material from both areas was approximately the same, it is conceivable that there could be $\sim 1500$ nM of weaker ligand with a conditional stability constant of $10^{10}$ in Cape Fear waters. A small portion of these ligands may be detected in our data since they would have an estimated $\alpha_{CuL}$ of $\sim 10^{12}$, near the lower limit of our detection window. However, given the large concentrations of ligands measured in the upper estuarine Cape Fear waters modeled with a fixed $K'_{CuL}$ of $10^{13.5}$, detectable weaker ligands would likely contribute only a small percentage (<10%) of the stronger ligand pool and would not significantly impact the observed distributions.

3.3. Estuarine distributions of TDCu

Concentrations of TDCu ranged from 3.2 to 22.7 nM and were always less than corresponding ligand concentrations within the experimental detection window (Table 1). Total dissolved Cu levels in the Cape Fear estuary are comparable to those in Galveston Bay (Tang et al., 2001), but relatively low compared with highly impacted estuaries such as San Francisco Bay (Donat et al., 1994), Severn Estuary (Apte et al., 1990), and harbors near Cape Cod (Moffett et al., 1997).

Correlations between TDCu and salinity (Fig. 7b) were generally weaker than for strong ligand ($\Sigma L_i$) and salinity in Cape Fear transects (Fig. 7a) with considerable variability in Cu concentrations in the upper estuary. The observed variability may be related to local anthropogenic sources including municipal runoff (i.e., city of Wilmington), shipping (Port of Wilmington), and industrial inputs occurring upstream of the Cape Fear estuary (Mallin et al., 1999b). Dissolved Cu concentrations may also be impacted by adsorption/desorption processes that occur within the turbidity maximum zone ($S < 5$) during early estuarine mixing (Sholkovitz, 1976) or recycling from sediment pore waters (Santschi et al., 1990). TDCu during April 1999 and June 1999 clearly exhibited non-conservative behavior, but similar patterns were not observed in $\Sigma L_i$ profiles (Fig. 7a). Therefore, variability in sources or natural processes does not impact $\Sigma L_i$ as strongly as TDCu. However, TDCu mixing plots (Fig. 7b) generally indicate that Cu entering the Cape Fear estuary can be effectively transported through the estuary possibly as a result of strong organic complexation (Van den Berg et al., 1987; Apte et al., 1990) enhanced by high river flows (Achterberg et al., 2002).

3.4. Estuarine distributions of DOC

The consistently conservative nature of DOM (quantified as DOC) in the Cape Fear estuary is evident in Fig. 7c (portion of DOC dataset from Avery et al., 2003). Again, river flow varied by as much as 10-fold during sampling trips but did not alter the conservative nature of the DOC profiles, although variations in river flow can impact freshwater DOC concentrations to some extent (Avery et al., 2003). Short residence times (typically <1 week under normal flow) and low primary productivity (Mallin et al., 1999a) limit the production of organic matter within the Cape Fear estuary. Furthermore, Avery et al. (2003) showed that <10% of the dissolved organic carbon entering this estuary is bioavailable for incubation periods of 1–3 months, much less than water residence times within this system. The facts that DOM in the Cape Fear is largely recalcitrant, dominated by freshwater inputs, exhibits conservative behavior over typical flow variations throughout the year, together strongly suggest that the DOM pool is dominated by riverine humic substances.

4. Discussion

4.1. Relationship between strong Cu ligands and DOM in the Cape Fear estuary

Strong Cu-complexing ligand concentrations were highly correlated with DOC concentrations at DOC levels $< 1000$ μM C ($r^2 = 0.93, p = 0.01$) for all sampling dates indicating riverine input of allochthonous organic matter is the primary source of strong Cu-complexing ligands to the Cape Fear estuary. Given the consistently conservative behavior

July 1998 (●)
y = -7.6x + 267
r² = 0.99

February 1999 (●)
y = -6.6x + 232
r² = 0.99

April 1999 (●)
y = -5.3x + 199
r² = 0.97

June 1999 (●)
y = -5.0x + 188
r² = 0.99

December 1999 (●)
y = -8.0x + 246
r² = 0.98

June 2000 (+)
y = -6.7x + 234
r² = 0.99

September 2000 (●)
y = -6.3x + 209
r² = 0.98

Theoretical mixing line
y = -6.0x + 213
r² = 0.93, p << 0.01

July 1998 (●)
y = -0.45x + 18.2
r² = 0.93

February 1999 (●)
y = -0.09x + 7.3
r² = 0.93

April 1999 (●)
y = -0.68x + 23.6
r² = 0.90

June 1999 (●)
y = -0.20x + 11.4
r² = 0.48

December 1999 (●)
y = -0.55x + 13.2
r² = 0.95

June 2000 (+)
y = -0.65x + 23.4
r² = 1.00

September 2000 (●)
y = -0.37x + 15.7
r² = 0.99
of both parameters in the middle and lower Cape Fear estuary (Fig. 7a and c), the close correlation is not surprising. At very high DOC concentrations (>1000 μM C), however, this same relationship does not hold. It could be argued that the data reveal a ligand source in the middle estuarine region, but there are no significant water inputs in this region and we have shown that sediments provide only a small quantity of ligands to Cape Fear estuarine waters (Shank et al., in press(b)). One likely explanation for the inconsistency could be procedural difficulties in determining an accurate sensitivity of the CSV titration data in these very organic-rich samples. Even though deposition times were short, surfactant effects cannot be discounted nor can the possibility that all of the natural ligands had been titrated in the sample so that a true sensitivity measurement was obtained (Kogut and Voelker, 2001, 2003; Voelker and Kogut, 2001). Two of the very high DOC concentrations were measured during June 2000 for which the titration data are illustrated in Fig. 5 (NAV—June 2000) and Fig. 6 (NCF6—June 2000). The linear ranges for both samples were deemed to include only the last 2–4 points, but in fact, it is possible that titration of the natural ligands was not yet completed before saturation of the HMDE occurred.

Another potential explanation for the variable relationship at high DOC levels is pH. All four samples that were not included in the linear DOC:ΣL correlation had pH values of 7.3–7.4 (see Table 1), whereas nearly all other samples exhibited pH values of 7.6–8.0, corresponding to a 2- to 5-fold difference in the quantity of H⁺ ions available to compete for binding sites. Since the functional groups responsible for strong Cu binding may contain some acidic characteristics (Averyt et al., 2004), as the pH decreased, binding sites would become occupied, especially in waters where Ca²⁺ and Mg²⁺ also compete for binding sites (Kogut and Voelker, 2001) as sea-
water mixing processes begin. To this point, however, there have been no direct measurements detailing the functional groups responsible for strong Cu complexation in organic-rich waters.

An empirical relationship between DOC and strong Cu ligand concentrations has been observed in other estuaries including the Severn estuary (Apte et al., 1990), Westerschelde and Oosterschelde estuaries (Gerringa et al., 1996), and Galveston Bay (Tang et al., 2001), but none with the strong correlation presented in this study. Gerringa et al. (1996) used a multiple regression fit with salinity and DOC to predict strong Cu ligand concentrations in the Westerschelde and Oosterschelde estuaries, but reported a correlation coefficient ($r^2$) of only 0.70. Unfortunately, Cu speciation data have not been reported for estuarine systems along the southeastern U.S. that have DOC concentrations similar to the Cape Fear (Moran et al., 1999).

### 4.2. Relationship between strong Cu ligands and DOM among ultrafiltered size fractions

Results of Cu speciation and DOC analyses on ultrafiltered samples are presented in Table 2. Recovery rates for DOC in the ultrafiltration procedure ranged from 99% to 115%, close to the margin of error in Cu speciation analyses and therefore not likely to significantly influence $\Sigma L_i$ results. The analytical detection window was consistent among fractions so that a similar range of ligand strengths was analyzed in each sample. Variations of the analytes among ‘colloidal’ (>1 and >10 kDa) and ‘truly dissolved’ (<1 kDa) sizes reveal that the strong Cu complexation capacity in the Cape Fear is distributed across a range of size fractions, including organic colloids. Sequestration in strong sulfide complexes or clusters (Rozan and Benoit, 1999; Luther et al., 2002) and binding by colloidal material are potential mechanisms that render Cu kinetically inert and therefore non-exchangeable in Cu speciation analyses (Kogut and Voelker, 2003). A more detailed analysis of the ultrafiltration results will be presented in a future manuscript. Pertinent to this manuscript is the linear

![Graph](image)

**Fig. 8.** Relationship between $\Sigma L_i$ and DOC concentrations for the Cape Fear estuary (February 1999 to September 2000). M18, M35, M54/61 (●), NAV (■), NCF6 (▲).

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Sample</th>
<th>Salinity</th>
<th>pH</th>
<th>DOC (μM C)</th>
<th>Detection window</th>
<th>$\Sigma L_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 2002</td>
<td>Feed</td>
<td>19</td>
<td>7.8</td>
<td>580</td>
<td>5.2</td>
<td>114 ± 8</td>
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<tr>
<td>Station B</td>
<td>10 kDa Perm</td>
<td>19</td>
<td>7.7</td>
<td>509</td>
<td>5.1</td>
<td>107 ± 5</td>
</tr>
<tr>
<td>(near M35)</td>
<td>1 kDa Perm</td>
<td>19</td>
<td>7.7</td>
<td>336</td>
<td>5.1</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>April 2002</td>
<td>Feed</td>
<td>29</td>
<td>7.9</td>
<td>219</td>
<td>5.1</td>
<td>49 ± 1</td>
</tr>
<tr>
<td>Station A</td>
<td>10 kDa Perm</td>
<td>29</td>
<td>7.9</td>
<td>198</td>
<td>5.1</td>
<td>24 ± 4</td>
</tr>
<tr>
<td>(near M18)</td>
<td>1 kDa Perm</td>
<td>29</td>
<td>7.9</td>
<td>141</td>
<td>5.1</td>
<td>3 ± 1</td>
</tr>
</tbody>
</table>

$\Sigma L_i$=mean±0.5 × range at fixed $K_{CuL}=10^{13.5}$, $n=2$. 
relationship between strong ligand ($\Sigma L_i$) and DOC concentrations among the ultrafiltered fractions (Fig. 9, $\Sigma L_i = 0.24\text{DOC} - 22$, $r^2 = 0.94$, $p \ll 0.01$) that is very similar to the observed estuarine transect data at DOC concentrations $< 1000 \mu\text{M C}$ (Fig. 8, $\Sigma L_i = 0.26\text{DOC} - 49$, $r^2 = 0.93$, $p \ll 0.01$). While strong Cu ligands existed as both ‘truly dissolved’ and ‘colloidal’ entities in Cape Fear waters, the strong Cu-complexation capacity was consistently a function of the quantity of organic material. Tang et al. (2001) also reported a relationship between DOC and strong Cu-complexing ligand concentrations among ultrafiltered size fractions in Galveston Bay, but did not provide regression analysis.

4.3. Strong Cu-complexation capacity of Cape Fear humics

Humic substances isolated from Cape Fear estuarine waters using solid phase C$_{18}$ extraction exhibited considerable strong Cu-complexation capacity (Table 3). The calculated detection window was approximately equal for all samples ($\log K_{\text{CuL}} = 5$) and equivalent to the estuarine samples (Table 1). A humic concentration of 26 mg l$^{-1}$ (sample #1, salinity = 5) produced a strong Cu-complexation capacity of 143 nM, equivalent to 5.5 nM mg$^{-1}$. Strong Cu ligand concentrations were linearly related to humic-derived DOC levels (Fig. 10, $r^2 = 0.92$, $p = 0.04$), but with variability at higher DOC concentrations as observed in the estuarine transects (Fig. 8). This variability supports the idea presented earlier that there could be a measurable competition effect for strong binding sites in low salinity waters due to pH influences in combination with Ca$^{2+}$ and Mg$^{2+}$ ions. Kogut and Voelker (2001) reported that strong Cu$^{2+}$ binding in SRFA might be affected by competition from Mg$^{2+}$ and Ca$^{2+}$, although some studies indicate otherwise (e.g., Hamilton-Taylor et al., 2002).

Humic C accounts for $\sim 30$–$50\%$ of the DOC in Cape Fear estuarine waters (R. Kieber, unpublished data). Using approximate estuarine DOC:humic C ratios of 3:1 and 2:1, all prepared humic solutions were projected to corresponding estuarine DOC concentrations assuming simple dilution and plotted with

![Fig. 9. Relationship between $\Sigma L_i$ and DOC concentrations in ultrafiltered size fractions of Cape Fear estuarine water (April 2002). Feed samples were 0.45 µm filtered.](image-url)
the corresponding $\Sigma L_i$ of the humic samples. These projections are supported by the approximate linearity of the DOC mixing profiles and by Avery et al. (2003) who reported consistently conservative behavior of DOC distributions in the Cape Fear regardless of normal fluctuations in river discharge or seasonality. Based on these projections, C$_{18}$ extracted humics may account for nearly 100% of strong Cu ligand concentrations typically observed in the Cape Fear estuary (Fig. 10). Additional evidence is provided by plots of Cu$^{2+}$ vs. CuT$^*$ (Voelker and Kogut, 2001) constructed for Cape Fear estuarine and humic samples (Fig. 11a–g). It is evident from these plots that the binding characteristics are quite similar at the detection window employed in this study when the range of Cu concentrations (CuT$^*$) were equivalent.

The strong Cu-complexation capacity of Cape Fear humics compares favorably with freshwater and seawater solutions of SRHA and SRFA isolates. Kogut and Voelker (2001) reported a 1 mg l$^{-1}$ seawater solution of SRHA produced $\sim$ 10 nM (log $K_{CuL}$ $\sim$ 12) strong Cu ligand (vs. $\sim$ 6 nM mg$^{-1}$ in this study). Xue and Sigg (1999) reported SRFA dissolved in freshwater at pH = 8.0 produced strong Cu ligands (log $K_{CuL}$ $\sim$ 14) at a 5 nM mg$^{-1}$ ratio. Both SRFA and SRHA extracts are prepared using XAD resin columns (International Humic Substance Society), suggesting C$_{18}$ and XAD extractions isolate similar types of Cu-complexing substances. Additionally, the Cape Fear River and Suwannee River have similar blackwater swamps in their headwaters that are the primary sources of these humics. Further supporting evidence for humics as primary Cu complexants was provided by Kogut and Voelker (2003) who showed that Cu complexation in estuarine waters near Cape Cod can be effectively modeled as concentrations of humic substances ranging from 0.3 to 0.7 mg l$^{-1}$.

In contrast to the similarities with SRFA and SRHA, the strong Cu-complexation ability of Cape Fear humics isolated by C$_{18}$ extraction differs considerably from humics isolated from seawater samples using the same technique. In separate studies, Van den Berg (1984) and Donat et al. (1986) reported C$_{18}$ columns isolated only 5–10% of the Cu-complexation capacity of oceanic waters. Van den Berg (1984) offered the possibility that Cu-bound ligands are retained by the C$_{18}$ column, whereas unbound ligands are not. Donat et al. (1986) postulated that marine organic Cu complexes must exhibit some degree of
hydrophilicity to not be retained by C18 columns. Data presented in this paper do not preclude either of these possibilities. However, the success of this study in using C18 extraction to isolate strong Cu-complexing ligands indicates there are significant chemical structural differences between strong ligands observed in the open ocean and in humic dominated estuaries such as the Cape Fear.

5. Conclusions

The organic-rich Cape Fear estuary exhibits high concentrations of very strong Cu-complexing ligands that are highly correlated with DOC levels (<1000 \mu M C) in estuarine samples and among ultrafiltered fractions of estuarine samples. Our results indicate allochthonous dissolved organic matter is the primary source of strong Cu-complexing ligands to the Cape Fear estuary. It may be possible to predict the strong Cu-complexation capacity of this humic-rich system by measuring its DOC content, especially in the middle and lower estuarine regions, because of short residence times and low primary productivity levels. Our data indicate that freshwater input of C18 extractable humic substances may account for all of the strong Cu ligands in the Cape Fear. This paper is the first to conclusively link humic substances extracted from an estuary to the strong Cu-complexation capacity measured directly within the same estuary. Allochthonous DOM may also be the primary source of strong Cu complexants in other organic-rich estuaries located along the southeastern U.S. coast, although there have been no detailed Cu speciation studies in these chemically similar environments.

Fig. 11. Plots of Cu^{2+} vs. CuT* for Cape Fear estuarine samples and humic samples. All estuarine samples are denoted by \( \times \) and humic samples denoted by \( \odot \).
Acknowledgements

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References


