THE ROLE OF BENTHIC MACROFAUNA IN INFLUENCING FLUXES AND SPECIATION OF DISSOLVED ZINC AND COPPER IN ESTUARINE SEDIMENTS

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ABSTRACT

Sediment flux experiments were carried out for sediment and water samples

collected on April 23, 2001 and June 26, 2001 from a site in the lower CFR estuary.

Benthic fluxes were determined for total dissolved copper (Cu) and zinc (Zn) and the

ligands that bind these metals. Benthic fluxes of total dissolved Cu (TDCu) ranged from

130 to -180 nmol·m^{-2}·d^{-1}, where a negative flux represents the migration of a species from

the sediment into the overlying water. The copper-complexing ligand fluxes ranged from

590 to -1030 nmol·m^{-2}·d^{-1}. Total dissolved Zn (TDZn) fluxes ranged from 56 to -300

nmol·m^{-2}·d^{-1} and the Zn-complexing ligand fluxes ranged from 1220 to -980 nmol·m^{-2}·d^{-1}.

Fluxes of both TDCu and TDZn were several times lower than the concentration of

metal-binding ligands, suggesting that both Cu and Zn are largely complexed when they

flux from sediments. There were no significant differences ($\alpha = 0.05$) between the two

seasons in the fluxes of TDZn and Zn- and Cu-complexing ligands. However, fluxes of

TDCu were significantly greater in April than in June.

The role of bioturbation in influencing benthic fluxes of these chemical species

was also investigated using *Streblospio benedicti,* an opportunistic species common in the

lower Cape Fear estuary. The presence of these polychaetes did not significantly affect

fluxes of metals or ligands in any of the experiments.

Speciation analysis using competitive ligand equilibration – cathodic stripping

voltammetry revealed that Cu was bound by a single strong class (L$_1$) whose K$_{cond}$ ranged

from $10^{13.5}$ to $10^{14.5}$, a result consistent with studies of Cu in this and other estuaries. Zn

speciation analyses revealed qualitatively that there are two separate ligand classes

responsible for binding dissolved zinc. The conditional stability constants of the two ligand classes are too close in value ($\sim 10^{7.5}$) to compute values for each ligand class.

ACKNOWLEDGEMENTS

DEDICATION

This thesis is dedicated to my parents, Ken and Una MacGillivray. Their faith,

patience, optimism, and wisdom helped replenish my well whenever it ran dry.

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INTRODUCTION

Background

The lower Cape Fear River is vital to southeastern North Carolina for several

reasons. Citizens and industries in the Cape Fear region use the river not only as a

consumable resource, but also as a sink for waste waters, both treated and untreated. The

river also hosts two important shipping terminals: the Port of Wilmington, one of the

state's two commercial ports, and the Military Ocean Terminal Sunny Point (MOTSU), a

military shipping terminal near Southport at the river's terminus. Point sources and runoff

water associated with agriculture, shipping, industry, and municipal development

ultimately drain into the Cape Fear River. Anthropogenic contaminants associated with

these activities can potentially degrade water quality and have deleterious effects on the

Cape Fear estuarine ecosystem.

Two of these potential contaminants are copper (Cu) and zinc (Zn), which are

widely used in numerous products associated with human and industrial activity. Zinc is a

key component of paint pigments, is added to animal feed and fertilizers, is present in

anti-fouling hardware for ships, and is the metal used for galvanization (Gupta and

Karuppiah, 1996; Comber et al. 2002; Heijerick et al., 2002). Copper is present in feeds

and fertilizers, wood preservatives, water tubing, and anti-fouling paints for ship and boat

bottoms (Seligman and Zirino, 1998). Metals such as Cu and Zn occur naturally at low

dissolved concentrations and are necessary trace nutrients for native biota. However, at

higher concentrations these metals can be toxic to organisms such as phytoplankton

(Sunda and Guillard, 1976; Brand et al., 1983; Brand et al., 1986) which form the base of

the food chain and support higher trophic levels in estuaries and the ocean.

Numerous studies have demonstrated the importance of metal speciation in controlling the toxicity, bioavailability, and geochemical behavior of trace metals such as Cu and Zn in estuarine and oceanic waters (see Bruland et al., 1991 and Donat and Bruland, 1995; and references therein). Speciation of a metal is defined as its distribution among various organic and inorganic forms, including inorganically complexed, organically complexed, and free hydrated ionic forms (Donat and Bruland, 1995). In many studies of estuarine waters, dissolved Cu speciation is dominated by at least two classes of organic ligands: a very strong class (L_1) which usually exists at relatively lower concentrations but with a large conditional stability constant (with respect to free Cu^{2+} ion, K') of 10^{11} to 10^{15}; and relatively weaker classes (L_2, L_3) with generally higher concentrations but smaller values of K' ($10^8 - 10^{10}$) (Apte et al. 1990; Moffett et al. 1990; Donat et al. 1994; Moffett, 1995). This large degree of complexation leads to virtually all total dissolved Cu (>99.9%) existing as relatively strong Cu-organic complexes in most estuarine and marine waters.

Zinc speciation in estuarine waters is less well known, primarily because there are fewer methods to characterize Zn-organic complexes in natural waters. However, in estuarine waters, one or two classes of ligands have been detected. These ligand classes have values of K' of $10^{7.4}$ to $10^{9.4}$ with concentrations of 40 to 160 nM (van den Berg and Dharmvanij, 1984; van den Berg et al. 1986, 1987; Muller and Kester, 1991; Gardner, 1999). Calculations of organically-complexed Zn in estuaries range widely between 1 and 95%, depending on ligand strength and concentration.

The exact sources of Cu- and Zn-complexing ligands, which constitute usually ≤0.1% of the total pool of dissolved organic carbon (DOC), are not well characterized. In

general, these ligands are composed of various colloidal or dissolved fractions of DOC, including phytoplankton or bacterial exudates and their degradation products (Anderson et al., 1984; Seritti et al., 1986; Kerner and Geisler, 1995; Moffett and Brand, 1996; Gordon et al., 2000) and humic substances (Kogut and Voelker, 2001).

Organic-rich estuaries such as the Cape Fear might be expected to contain relatively high concentrations of metal-complexing ligands, given that DOC concentrations typically range from 200-800 μM (Avery et al., 2002). Recent studies in the Cape Fear estuary (CFE) indicate that concentrations of strong (L_1-type) Cu-complexing ligands (10-100 μM) are in large excess relative to total dissolved Cu concentrations (5 -10 nM), resulting in >99.9 % of the TDCu existing as strong organic complexes (Shank et al., 2003).

Zinc speciation has never been determined in the CFE. However, O'Connell (1999) determined total dissolved Zn concentrations throughout the lower CF system over various seasons and hydrologic conditions. Concentrations ranged from 20-90 nM in the upper estuary (low salinity) to 15-60 nM in the middle and lower estuary.

Because the organic ligands that complex Cu and Zn have an important influence on their behavior in estuarine systems such as the Cape Fear, an understanding of the source of these ligands is clearly desirable. Most previous work on Cu and Zn speciation in estuaries has focused on concentrations of complexing ligands in surface waters, therefore reflecting primarily riverine and oceanic water column processes. However, previous work has shown that *sediments* can be important sources of ligands to the overlying water column. For example, Skrabal et al. (1997) showed that fluxes from bottom sediments may be a significant source of Cu-complexing ligands to overlying

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waters of Chesapeake Bay. Depth profiles of sediment porewaters in Chesapeake Bay show that sediments are a potentially large reservoir of Cu-complexing ligands (Skrabal et al., 2000). Studies in the Cape Fear estuary by Shank et al. (2003) also suggest that benthic fluxes may contribute a small fraction of Cu-complexing ligands to overlying waters. Preliminary studies by van den Berg and Dharmvanij (1984) suggest that sediment porewaters are enriched in Zn-complexing ligands relative to overlying waters. These findings support the assertion that sediments can act as sources of metal-binding ligands. Also, it is well documented that metals such as Cu and Zn that accumulate in sediments underlying a body of water can be a source of metals to the water column (Elderfield et al., 1981; Westerland et al., 1986; Kuwabara et al., 1996). Hence benthic fluxes represent a potentially important source of dissolved Cu and Zn and their complexing ligands to the Cape Fear estuary and estuarine systems in general.

As illustrated in Fig. 1, there are several processes and routes by which metals such as Cu, Zn, and their complexing ligands enter the estuary. This work focuses on the contribution from benthic fluxes. While simple diffusion from sediments arising from concentration gradients can engender fluxes of ligands, metals, or both, it is reasonable to investigate whether or not the biologically-induced disturbance of sediments might also cause such fluxes. The activity of benthic (bottom-dwelling) organisms has been shown previously to affect the sediment-water exchange of nutrients and trace metals such as iron, manganese, and arsenic (Allen, 1982; Riedel et al. 1987, 1997). Numerous benthic organisms inhabit the Cape Fear River estuary sediments (Mallin et al., 1996), and their feeding and burrowing activities may influence fluxes of materials. Across the sediment-water interface.
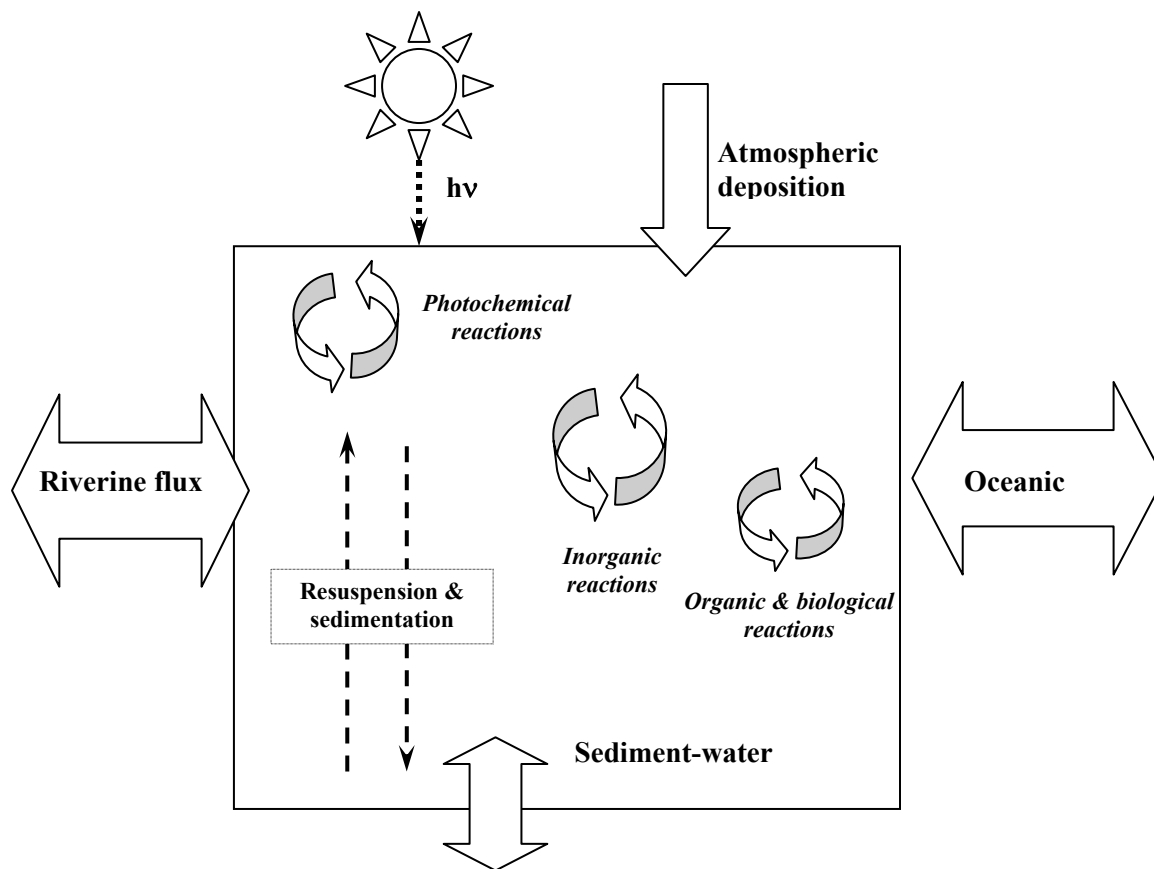
Fig. 1. Illustration of the estuarine box model. This figure shows the possible fates and origins of trace species such as zinc, copper, and metal-complexing ligands.

One such organism is *Streblospio benedicti,* a polychaete common in the Cape Fear River estuary that can quickly alter the topography of the sediment surface. *S. benedicti* is an infaunal organism, typically 20 mm or less in length. It feeds by burrowing into the sediment and then building a feeding tube that extends through the sediment to the overlying water (Levin, 1981, 1984). Levin (1981) reports that the population density of *S. benedicti* in Mission Bay in San Diego, California is 4,000 to 6,000 individuals/m^2; a similar density of *S. benedicti* often exists in the lower Cape Fear River estuary (oral communication, M. Posey, 2002). This species can be the dominant polychaete in the lower estuary (Posey and Alphin, 2001). The role of this organism in affecting amino acid diagenesis has been investigated previously in Cape Fear sediments (Thomas, 1998). The experiments described in this thesis are the first to explore the possible role that bioturbation may play in sediment fluxes of Cu and Zn and the ligands that bind them.

Goals

The study presented in this thesis has the following goals:

- Core incubation experiments are performed to determine benthic fluxes for total dissolved Cu, total dissolved Zn, and the ligands that bind these metals.

- Addition of the polychaete *S. benedicti* to incubated cores will determine the effect bioturbation has on benthic fluxes of these two metals and their ligands.

- The concentration of Zn in the Cape Fear River estuary will be measured, and speciation data for Zn will be presented for the first time.

METHODS

Sediment and Water Collection

All equipment and sample bottles (high density polyethylene (HDPE) for total dissolved metals; fluorinated HDPE (FHDPE) for speciation samples) were subjected to a stringent cleaning regimen in order to minimize contamination. All equipment was soaked for one week in each of the following: Micro or Citranox detergent, 3 M HCl, 2 M HNO_3, and finally ~0.01 M trace metal grade HCl (Fisher). The first three soaking solutions were prepared using deionized water (DIW) and all bottles were rinsed well with DIW after these treatments. The final solution was prepared using ultrapure deionized water from a Milli-Q system (Millipore; ≥ 18 mΩ cm^{-1}). FEP-Teflon bottles (60 mL) used for speciation analysis were soaked in warm concentrated HNO_3 for several weeks, rinsed with DIW, then soaked in pH 2 trace metal grade HCl in MQ. Acrylic core tubes used for sediment collection were soaked for several days in 1 M HCl and rinsed with DIW.

Water and sediments were collected from the lower Cape Fear River estuary in the vicinity of channel marker 23 (Fig. 2), a site frequented by marine vessels as they visit the port facility at Military Ocean Terminal Sunny Point (MOTSU). Sampling was conducted on two different dates: April 23, 2001 and June 26, 2001. Samples were collected from aboard the R.V. *Cape Fear* in 3-5 m water depth with the use of a crane-mounted box corer. The box corer was sunk several times until a sample was retrieved that was leaking neither sediment nor water. This inspection ensured that that the samples were representative, undisturbed cross sections of the sediment in the shallow portion of the estuary. Six individual core samples were collected from the stainless steel

Fig. 2. Map showing the lower estuary sampling site near channel marker 23.

box by inserting acrylic tubes (40 cm height, 14 cm diameter) into the sediment in the box. Approximately 25 cm of sediment was collected in each core. The cores were handled with polyethylene gloves.

Cores were sealed at the bottom using polyethylene caps. Cores were transported in a polyethylene crate to an environmental chamber where the flux experiments were conducted. Unfiltered bottom water was also collected in a 50-L polyethylene carboy at the site using Teflon-lined polyethylene sample tubing and an air-operated polyethylene pump. The water collected from the sampling site on 4/23/01 had salinity of 24, pH of 7.8, and temperature of 20^0C. The water collected from the sampling site on 6/26/01 had salinity of 21, pH of 8.0, and temperature of 27^0C.

Core Incubation Experiments

Acrylic lids were fixed to the tops of the cores, sealing them against contamination and providing parts for sampling and aeration. Prior to the experiment, cores were gently drained of their overlying water and flushed with three volumes of bottom water. The volume of water overlying the core was finally set at 15 to 18 cm above the sediment-water interface. The temperature of the environmental chamber was set to match that of the sampling site and cores were kept dark to simulate ambient conditions. To each of three of the six cores were added ~30 *S. benedicti* individuals, to achieve a density of ~2,000 organisms per square meter. These three cores were the "seeded" cores, and the other three cores were the "unseeded" cores. Filtered (0.45 μm) air was gently bubbled into each of the core tubes to maintain aerobic conditions similar to those in the estuary at the time of sampling (Fig.3).

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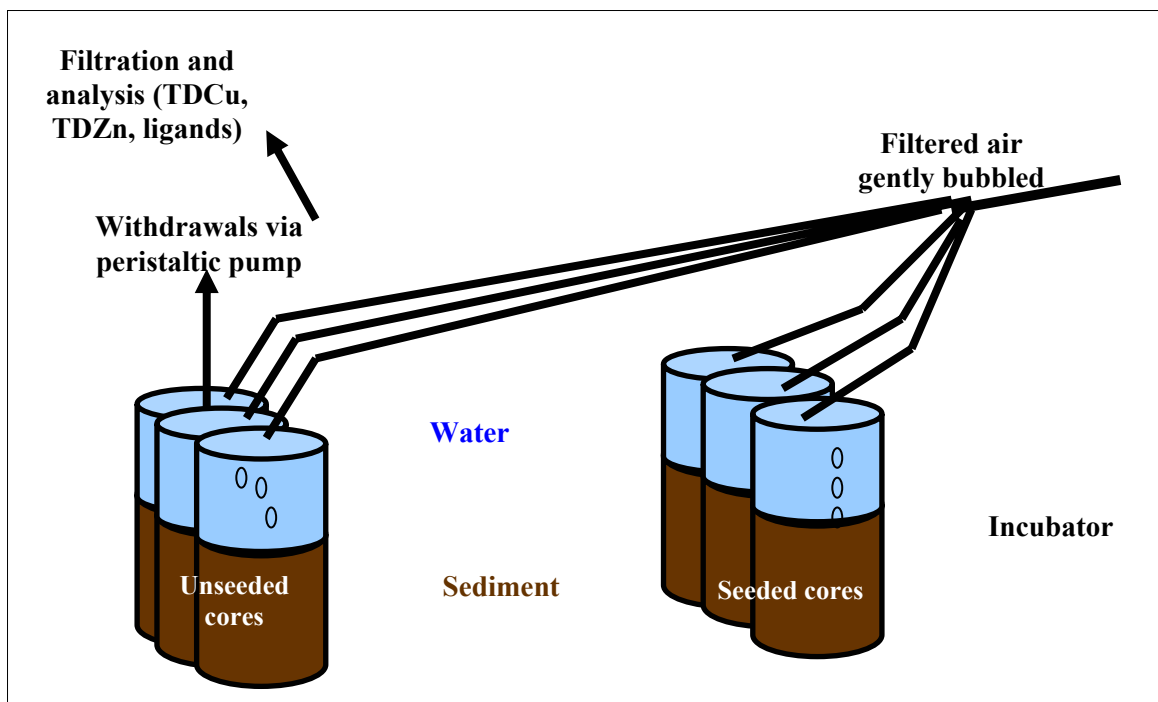
Fig. 3. Diagram of apparatus used in core incubation experiments.

Water was then withdrawn using a Masterflex peristaltic pump using trace-metal clean C-Flex (Cole-Parmer) tubing and plastic connectors and spigots. The water was filtered with 47 mm, 0.2 μm Supor (Pall Gelman) polyethersulfone filters before being collected in acid-washed HDPE bottles for total dissolved metal (TDM) analysis and acid-washed FHDPE bottles for speciation analysis. Approx 100 ml of water was collected for TDM analysis and 300 ml was collected for speciation experiments. These water samplings constitute the "T=0" time point for each analysis conducted. Water levels in the cores were recorded to the nearest 0.1 cm before and after the water collection. As a control, bottom estuary water that was collected along with the core samples was also filtered and sampled for analysis. This water was kept separately in a large trace-metal clean polyethylene carboy, and is referred to as "carboy sample" or "carboy water" in this thesis.

After 48 hours (April 23) or 60 hours (June 26) had elapsed, the cores were again sampled using the aforementioned procedure. These samples constitute the "T=48" (April 23) or "T=60" (June 26) time point for each of the analyses conducted. A control sample was filtered and collected from the carboy as well at this time. All speciation samples were double-bagged and immediately frozen and kept at -10° C. All total dissolved metal samples were acidified as described below.

Total Dissolved Metal Determinations

Each TDM sample was acidified with 12 M ultrapure HCl (Fisher Optima) to pH ~2 to prevent absorption of dissolved metal onto the plastic container walls. Samples were UV-irradiated for 6 hours using a 1.2 kW Hg arc lamp (Ace Glass) in order to

destroy any organic matter that could act as a source of metal-binding ligands or surfactants.

UV irradiation of each sample was performed in a 100-ml trace metal clean Teflon beaker supported by a Pyrex petri dish; this assembly was in turn covered by a quartz beaker. Water lost during irradiation was replaced with Milli-Q water. Irradiated samples were allowed to sit for 48 h before electrochemical analysis in order to minimize the possibility that the sample would still contain any reactive, unstable radicals that can form during the UV irradiation. We have found that such species can sometimes interfere with electrochemical analysis.

The majority of samples were analyzed electrochemically using an E.G.&G. model 303A electrochemical stand operated in the hanging mercury drop electrode (HMDE mode) and equipped with a saturated calomel reference electrode, platinum wire counter electrode, and model 305 magnetic stirrer. The HMDE was interfaced with an Autolab PGSTAT-10 potentiostat and controlled using Autolab software running on a Dell computer.

Total dissolved (TD) copper was determined using a modification of the adsorptive cathodic stripping voltammetry method of van den Berg (1986), which employs 8-hydroxyquinoline as the adsorptive chelator. Typically 5-ml aliquots of sample were used for analysis. Prior to analysis, acidified samples were partially neutralized by using 5 M ultrapure NH_4OH (Fisher Optima) before buffering to the optimal method pH using N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES). Analyses were conducted using square wave modulation. Concentrations were quantified by the method of standard additions. A standard reference material was

analyzed to ascertain the effectiveness of the TD Cu method. The analysis of CASS-4

standard reference material (coastal seawater) that contained 9.3 ± 0.9 nM TD Cu was

analyzed by the above methods and found to contain 9.6 ± 0.7 nM TD Cu (n = 3).

Total dissolved Zn was determined on UV-irradiated samples using a

modification of the adsorptive cathodic stripping voltammetric technique of van den Berg

(1984 a) which utilizes ammonium pyrrolidinedithiocarbamate (APDC) as the adsorptive

chelator. UV-irradiated samples were partially neutralized using 5 M ultrapure NH_4OH

before being buffered to optimal analytical pH using N-(2-Hydroxyethyl)piperazine-N'-

(3-propanesulfonic acid) (HEPPS). Zinc concentrations were quantified as described for

Cu. SLEW-3 (estuarine water) standard reference material was analyzed in order to

ascertain the effectiveness of the TD Zn determination method just described. The

analysis of the SLEW-3 indicated that its [TDZn] was 3.5 ± 0.5 nM n = 4). This value is

within the range of the actual concentration (3.1 ± 0.6 nM). Analytical details of the Cu

and Zn determinations are shown in Table 1.

	Copper	Zinc
Voltammetric Method	Square wave	Differential Pulse
Conditioning Potential (V)	0	-1.3
Duration (s)	0	20
Deposition Potential (V)	-1.1	-0.8
Duration (s)	30	20
Equilibration Time (s)	5	5
Frequency (Hz)	50	50
Stirring	Fast	Slow
Initial Pot (V)	-0.2	-0.95
End Pot (V)	-0.65	-1.175
Step Pot (V)	.0021	.0021
Amplitude	.025	.025
Ligand	8-hydroxyquinoline	APDC
Concentration	50 mM	1 mM
buffer	HEPES, 0.01 M	HEPPS, 0.01 M
NH_4OH	Added to pH = 7.8	Added to pH = 7.6
Purge with N_2	4 min	4 min

Table 1. Instrumental parameters and reagent concentrations used for the total dissolved metal determinations of Cu and Zn

Copper and Zinc Speciation Experiments

Both Cu and Zn speciation titrations were carried out using competitive ligand equilibration – cathodic stripping voltammetry (CLE-CSV) techniques. The theory and application of this technique has been described in detail in many previous studies (van den Berg, 1984 b; Donat et al., 1994) and are described only briefly here. Each sample was thawed and buffered to the appropriate in situ pH using HEPES (for Cu) and HEPPS (fOR Zn) at a final buffer concentration of 0.01 M. Each sample was then distributed in 5.5-mL aliquots into 24 20-ml Teflon bottles to create 2 duplicate series of 12 bottles per series. Each series of bottles was then spiked with incrementally increasing concentrations of Cu or Zn using the appropriate metal standard. For the Cu speciation analysis, the schedule of additions ranged from 0 added nM Cu to 190 added nM Cu. For the zinc speciation analysis, the schedule of additions ranged from 0 added nM Zn to 665 added nM Zn. Analytical details of the Cu and Zn speciation determinations are shown in Table 2.

Once the sample had been spiked with metal standard, it was left undisturbed for 4 h in order to allow the natural ligands to come to equilibrium with the added metal. At the conclusion of this 4 h period, the competitive ligand was added to each series. Samples were left undisturbed for a 12-hour period in order to allow the natural and added ligands to reach a competitive equilibrium with the metal. For the Cu speciation experiments, 8-hydroxyquinoline (8-HQ) was used as the competing ligand, whereas APDC (defined previously) was used for Zn speciation determinations. The Cu-8HQ or Zn-APDC complex that is formed can be detected electroanalytically. Therefore, when Cu-8HQ or Zn-APDC complexes compete with naturally occurring strong Cu- or Zn-

complexing ligands, an electrochemical signal can be detected which results from the reduction the Cu-8-HQ or Zn-APDC complexes. When the added ligand completely outcompetes the natural ligands, the titration curve becomes linear. Because the concentration and strength of the added ligand is known, the analysis of an entire titration series can yield the concentration and strength (conditional stability constant, $K^{'}$) of the natural ligands present that bind the metal. Linearization procedures (van den Berg, 1982; Ruzic, 1982) are used to determine these parameters for the Cu- and Zn-binding ligands in these CFE samples.

Free Cu^{2+} and Zn^{2+} concentrations were then determined using equilibrium calculations (van den Berg et al., 1990). The ligand used for detecting Cu has the capability of detecting that fraction of Cu-complexing ligands which are very strong, with $K' \geq 10^{13}$ (van den Berg and Donat, 1992). Shank et al. (2003) found that the strong ligands are in large excess relative to total dissolved Cu in the CFE and play the dominant role in affecting its speciation. The competitive Zn ligand, APDC, is capable of detecting ligands with $K' = 10^7 - 10^{10}$; this is the only ligand currently available for electroanalytical studies for Zn.

	Copper	**Zinc**
Voltammetric Method	Square wave	Differential Pulse
Conditioning Potential (V)	0	-0.3 V
Duration (s)	0	20
Deposition Potential (V)	-0.2	-0.8
Duration (s)	75	20
Equilibration Time (s)	5	0
Stirring	Fast	slow
Initial Pot (V)	-0.25	-0.95
End Pot (V)	-0.65	-1.175
Step Pot (V)	0.002	.0021
Amplitude	.025	.05
Ligand	8-hydroxyquinoline	APDC
Concentration	1.2 μM	40 μM
buffer	0.01 M HEPES	0.01 M HEPPS
NH$_4$OH	Added to pH = 8	Added to pH = 8
Purge with N$_2$	4 min	4 min

Table 2. Instrumental parameters and reagent concentrations used in the CLE-CSV titrations of Cu and Zn

RESULTS AND DISCUSSION

Fluxes of Total Dissolved Metals

The overall range of TDCu in these experiments was 5 to 11 nM, which is well within the range of water-column concentrations found by Shank et al. (2003) in the CFE. Fluxes of total dissolved Cu ranged from 130 to -180 $nmol \cdot m^{-2} \cdot d^{-1}$ for both sampling periods, where negative values denote a net flux of ligands from the sediment to the overlying water (Figure 4 and Table 3). These are similar to the fluxes determined by Shank (2003), also carried out in the lower CFE. Tables 4 (concentrations) and 5 (fluxes) show TDCu data for similar estuarine environments. Fluxes ranged from 22 to -180 $nmol \cdot m^2 \cdot d^1$ for the April 2001 experiments to 130 to -24 $nmol \cdot m^{-2} \cdot d^{-1}$ for June 2001. The range of fluxes is well within that found in many coastal and estuarine environments, as summarized by Rivera-Duarte and Flegal (1997) who found that 73% of measurements in such systems fell within the range of ± 300 $nmol \cdot m^{-2} \cdot d^{-1}$. A one-tailed Mann-Whitney test (Zar, 1984) was used to ascertain that the April fluxes were greater than the June fluxes for TD Cu (95% confidence level). One possible explanation for lower fluxes in June 2001 would be the retention of Cu in trace sulfide phases during the warmer months when oxygen utilization is increased by higher rates of organic matter decomposition (Skrabal et al., 2000).

Calculations based on the range of fluxes, the residence time of water in the CFE, and an average overlying-water TDCu value of 5-11 nM were performed to estimate the contribution that sediments make to the TDCu in the overlying water. These calculations
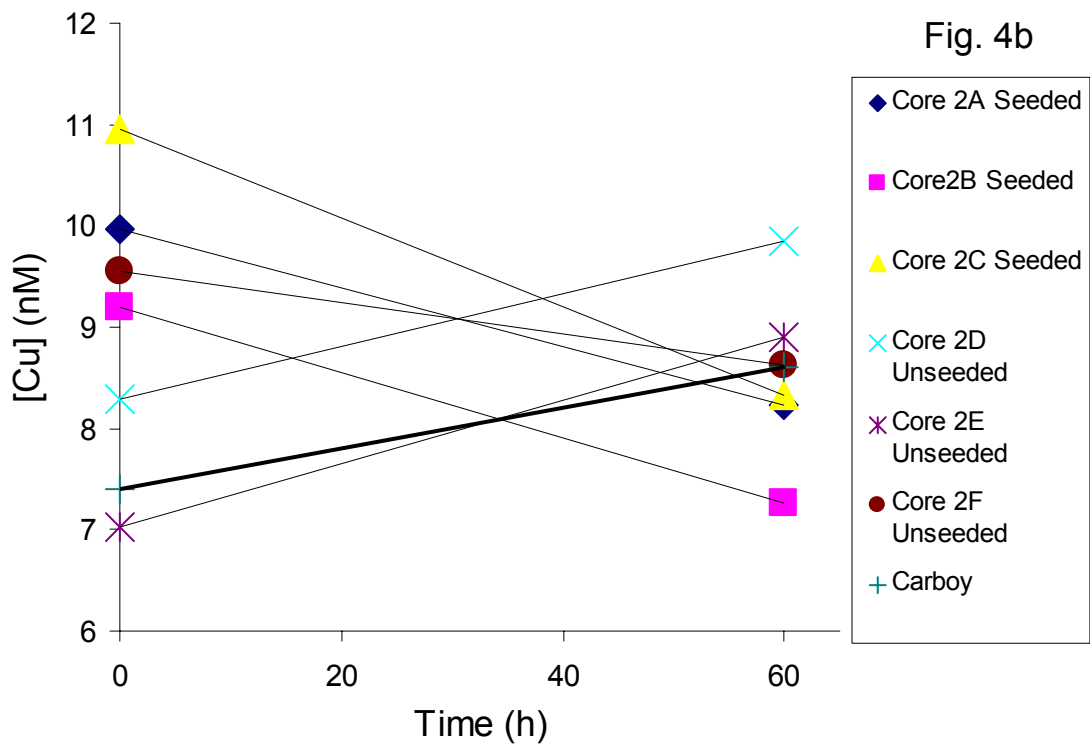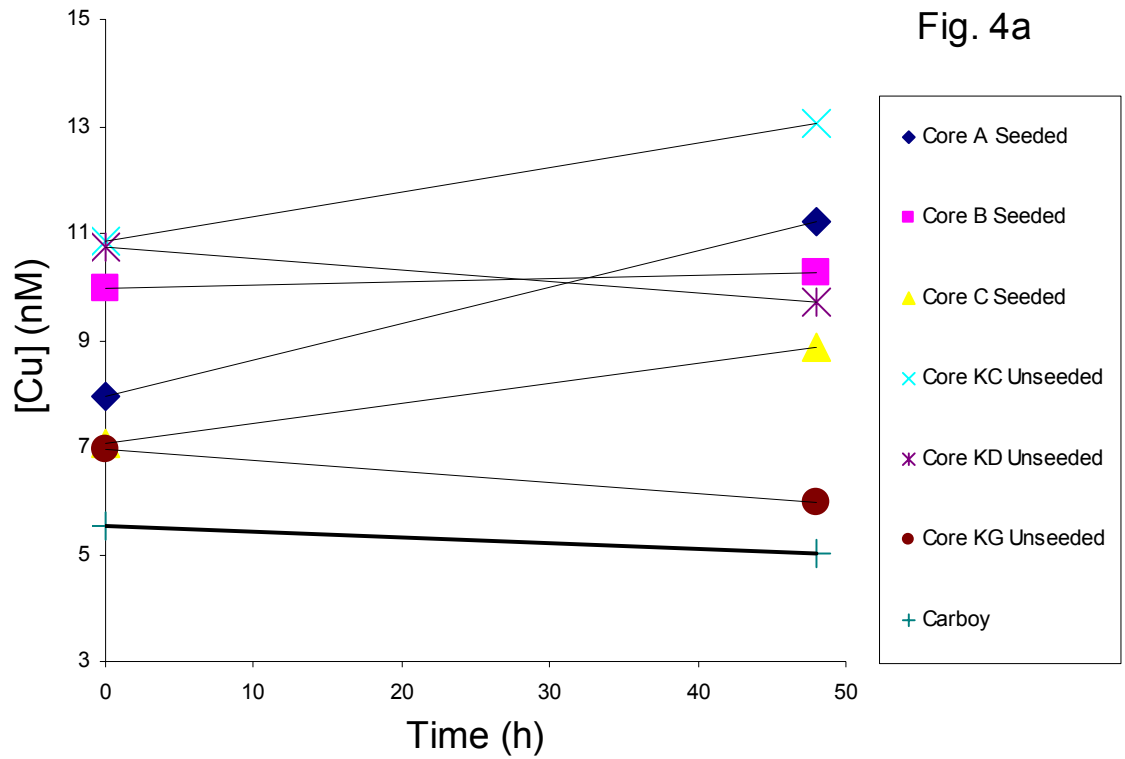
Figure 4. Concentration of total dissolved copper vs. time for six cores during (a) the April 23, 2001 and (b) the June 26, 2001 sediment flux experiments.

Sample	Td [Cu] (nM)		Flux (nmol·m^{-2}·d^{-1})
	T = 0	T = 48	
Carboy	5.5 ± 1.2	5.0 ± 0.0	-
Core A seeded	8.0 ± 0.6	11.2 ± 0.7	-180
Core B seeded	10.0 ± 0.9	10.2 ± 0.5	-39
Core C seeded	7.1 ± 1.2	8.9 ± 0.9	-110
Core KC unseeded	10.8 ± 1.9	13.0 ± 0.9	-110
Core KD unseeded	10.8 ± 1.2	9.7 ± 1.0	24
Core KG unseeded	7.0 ± 0.7	6.0 ± 1.1	22

a.

Sample	Td [Cu] (nM)		Flux (nmol·m^{-2}·d^{-1})
	T = 0	T = 60	
Carboy	7.4 ± 0.9	8.6 ± 1.1	-
Core 2A seeded	10.0 ± 1.7	8.2 ± 0.6	120
Core 2B seeded	9.2 ± 0.7	7.3 ± 1.0	140
Core 2C seeded	11.0 ± 0.6	8.3 ± 1.1	160
Core 2D unseeded	8.3 ± 0.7	9.8 ± 1.4	-14
Core 2E unseeded	7.0 ± 1.8	8.9 ± 0.6	-31
Core 2F unseeded	9.6 ± 1.5	8.6 ± 0.0	87

b.

Table 3. Total dissolved copper concentrations and fluxes for the (a) April 23, 2001 experiment and the (b) June 26, 2001 experiment. Negative fluxes denote a net departure of ligands from the sediment to the overlying water. Values are based on the mean of two replicate trials. The reported error = (|difference in trials |÷ 2).

Total Dissolved Copper Concentrations in Estuaries (nM)					
Sheldt (1,3)	Sabine,TX (2)	San Francisco Bay (4)	Tamar (5)	Ribble(5)	Cape Fear (this study)
10-20	10-15	27-30	40	25	5-11

a.

Copper-Complexing Ligand Concentrations in Estuaries (nM)				
Scheldt (1)	Chesapeake Bay (6)	Tamar (5)	Ribble (5)	Cape Fear (this study and Shank, 2003)
120	45	200	500	70 - 100

b.

Table 4. Concentrations of (a) total dissolved copper and (b) Cu-complexing ligand in selected estuarine environments. (1) van den Berg et al. (1987) (2) Benoit et al. (1994) (3) Zwolsman et al. (1997) (4) Flegal et al. (1991) (5) van den Berg et al. (1986) (6) Skrabal et al. (1997)

Total Dissolved Zinc and Total Dissolved Copper Fluxes in Estuaries (nmol·m^{-2}·d^{-1})		
Location	TDCu	TDZn
Bay of Cadiz [1]	-14 to -411	-
Galveston Bay [2]	-	-2400
Chesapeake Bay [3]	-11 to -85	-
Chesapeake Bay 1994 [4]	-610	-
San Diego Bay 2001 [5]	-1300 to -4200	-1500 to -31,100
Delaware Bay [6]	1200	-

Table 5. Comparison of TDCu and TDZn fluxes for different estuarine environments. Negative values indicate a flux out of the sediment into the overlying water. Sources: (1) Blasco et al. (2000); (2) Warnken et al. (2000); (3) Skrabal et al. (1997); (4) Riedel et al. (1997); (5) Anderson et al. (2001); (6) Church (1986)

indicate that benthic fluxes may supply from 0.2% to 5.6% of the TDCu in the overlying waters, suggesting benthic fluxes supply very little TDCu to the overlying water. (This and subsequent percent standing stock calculations in this study are based on a water residence time of 7 to 14 days for the Cape Fear estuary and an average water depth of 6 meters.) A two-tailed Mann-Whitney test (Zar, 1984) was used to determine that there were greater fluxes in the sediment cores without *S. benedicti* than those that were seeded with the organisms (90% confidence level) for the June experiment only. There was no difference between seeded and unseeded cores in the TDCu fluxes for the April tests.

Total dissolved Zn concentrations ranged from 5 to 10 nM (Figure 5 and Table 6). Earlier work in the lower CFE by O'Connell (1999) found concentrations from 15 to 45 nM, similar to concentrations observed at similar estuarine sites (Table 7). Total dissolved Zn fluxes varied from 56 to- 300 nmol·m^{-2}·d^{-1} over both sampling periods. Fluxes generally decreased from April to June, though a Mann-Whitney test revealed that there was no statistical difference (95% confidence level) between the two periods. There is no basis of comparison available for Zn flux experiments in the CFE. However, the results of Zn flux studies carried out at several different estuaries are summarized in Table 5. Note the Galveston Bay and San Diego Bay estuaries are both impacted sites and large fluxes from these sediments are observed. Based on a overlying water TDZn concentration of 30 nM (O'Connell, 1999), the contribution of sediment fluxes to the standing stock of TDZn in the estuary's overlying waters is computed to be only 0.2% to 2.6% of the TDZn in the overlying waters, suggesting that sediments do not supply an important fraction of the estuary's TDZn. There was no difference (90% confidence level) between the TDZn fluxes of seeded and unseeded cores for either season.
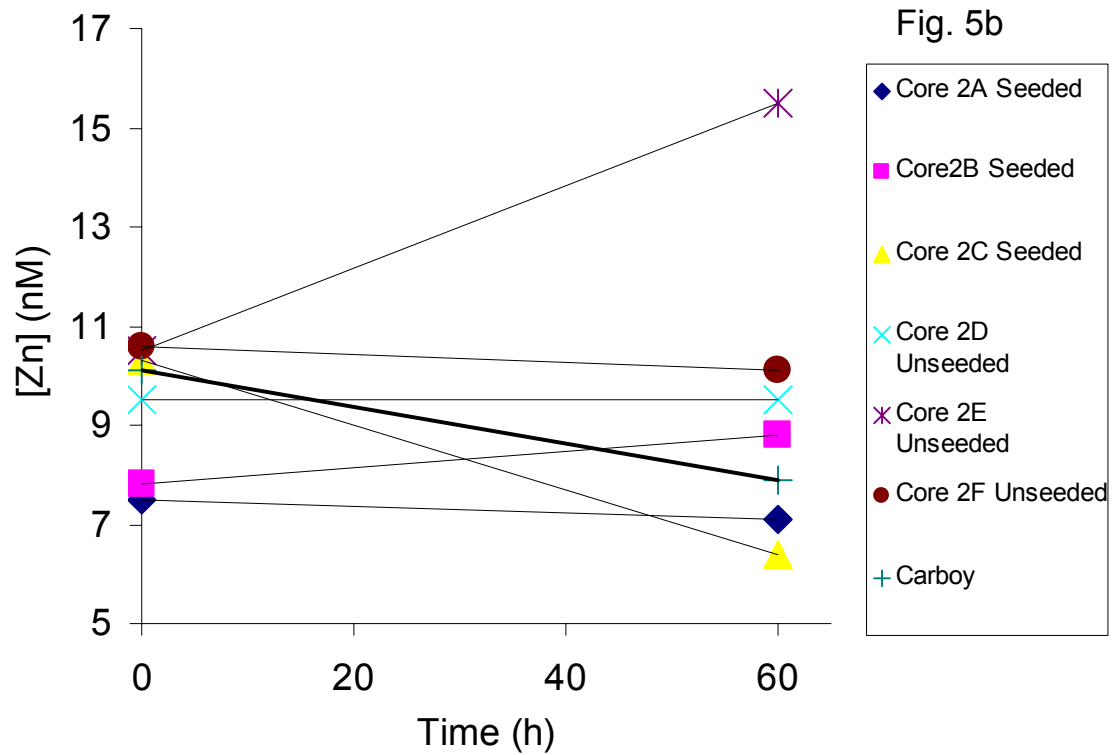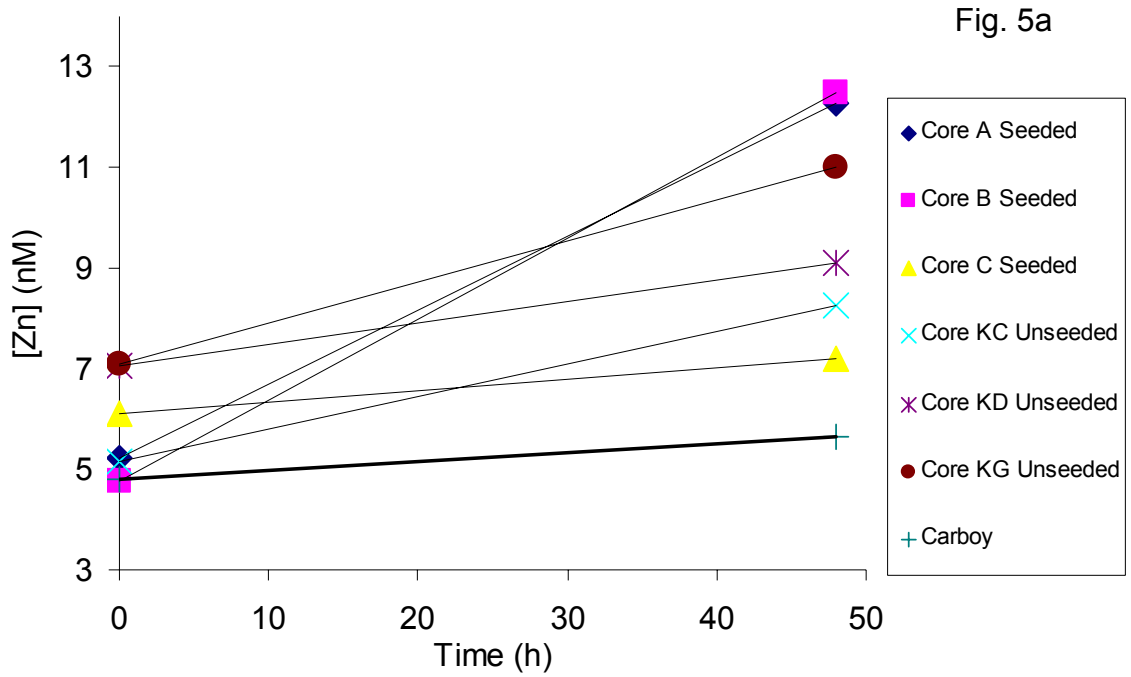
Figure 5. Concentration of total dissolved zinc vs. time for six cores during (5a) the April 23, 2001 and (5b) the June 26, 2001 sediment flux experiments.

Sample	Td [Zn] (nM)		Flux
	T = 0	T = 48	(nmol·m^{-2}·d^{-1})
Carboy	4.8 ± 0.4	5.6 ± 1.1	-
Core A seeded	5.2 ± 1.2	12.3 ± 1.0	-300
Core B seeded	4.8 ± 0.8	12.5 ± 2.0	-340
Core C seeded	6.1 ± 1.3	7.2 ± 0.9	-13
Core KC unseeded	5.2 ± 0.5	8.3 ± 1.5	-97
Core KD unseeded	7.1 ± 0.2	9.1 ± 0.2	-56
Core KG unseeded	7.1 ± 0.9	11.0 ± 1.7	-140

a.

Sample	Td [Zn] (nM)		Flux
	T = 0	T = 60	(nmol·m^{-2}·d^{-1})
Carboy	10.1 ± 1.0	7.9 ± 2.6	-
Core 2A seeded	7.5 ± 1.1	7.1 ± 0.9	-58
Core 2B seeded	7.8 ± 2.1	8.8 ± 0.1	-115
Core 2C seeded	10.3 ± 2.1	6.4 ± 0.6	56
Core 2D unseeded	9.5 ± 1.8	9.5 ± 1.06	-77
Core 2E unseeded	10.5 ± 1.7	15.5 ± 2.2	-245
Core 2F unseeded	10.6 ± 1.2	10.1 ± 1.8	-54

b.

Table 6. Total dissolved zinc concentrations and fluxes for the (a) April 23, 2001 experiment and the (b) June 26, 2001 experiment. Negative fluxes denote a net flux of ligands from the sediment to the overlying water. Values are based on the mean of two replicate trials. The reported error = (|difference in trials |÷ 2).

Total Dissolved Zinc and Zinc-Ligand Concentrations (nM)		
Location	[TDZn]	[Zn-L]
Scheldt [1]	10	50
Scheldt [2]	10-20	10-50
Sabine [3]	10-15	15-20
SanFrancisco [4]	27-30	6-12
Tamar [5]	40	-
Humber [6]	-	120-150
Ribble [5]	25	-
Galveston Bay [7]	-	10-13
Cape Fear [8]	5-45	36-92

Table 7. Concentrations of total dissolved Zn and Zn-complexing ligand in selected estuarine environments. (1) van den Berg et al. (1987) (2) Zwolsman et al. (1997) (3) Benoit et al. 1994 (4) Flegal et al. (1991) (5) van den Berg et al. (1986) (6) Gardner 1999 (7) Warnken et al. (2000) (8) this study and O'Connell (1999, TDZn only)

In light of the fact that only the June 2001 TDCu fluxes were affected by the presence of *S. benedicti*, it is interesting to note the results of treating all of the TDM analyses as experiments consisting of 6 replicate cores. When this assumption is made, there is a statistically significant (95% CL, two-tailed nonparametric test, Zar, 1984) flux of metals out of the sediments in both of the TDZn experiments (April and June 2001). The legitimacy of this assumption can be reviewed when future bioturbation experiments are carried out, presumably with a greater number of replicates per experiment.

Cu- and Zn-Complexing Ligand Fluxes

Copper-binding ligand concentrations were determined from the speciation experiments. Concentrations of Cu-complexing ligands ranged from 75 to 96 nM in April and 69 to 101 nM in June (Figure 6 and Table 8). This range of concentrations is well within the range found by Shank et al. (2002). Fluxes of strong Cu-complexing ligands ranged from 590 to -1000 nmol·m^{-2}·d^{-1}. There was no statistical difference in Cu-L fluxes of the two sampling periods (95 % confidence level), nor was there any difference in the fluxes of seeded and unseeded cores (90% confidence level). In separate work on the CFE , Shank et al. (2002) found no Cu-L flux in April, and a flux of -130 nmol·m^{-2}·d^{-1} for the June sampling period.

Zinc ligand concentrations ranged from 36 to 92 nM. Values in April ranged from 36 to 80 nM, and from 50 to 92 in June (Figure 7 and Table 9). These values are comparable to the 40-160 nM [Zn-L] reported by van den Berg et al. (1986) in the Tamar estuary. Zinc ligand fluxes ranged from 1200 to -980 nmol·m^{-2}·d^{-1}. There was no statistical difference (confidence level=95%) between the fluxes determined for the two different experiment dates, nor was there any difference (confidence level = 90%) in the

Fig. 6a

Legend:
- Core A Seeded
- Core B Seeded
- Core C Seeded
- Core KC Unseeded
- Core KD Unseeded
- Core KG Unseeded
- Carboy

Fig. 6b

Legend:
- Core 2A Seeded
- Core 2B Seeded
- Core 2C Seeded
- Core 2D Unseeded
- Core 2E Unseeded
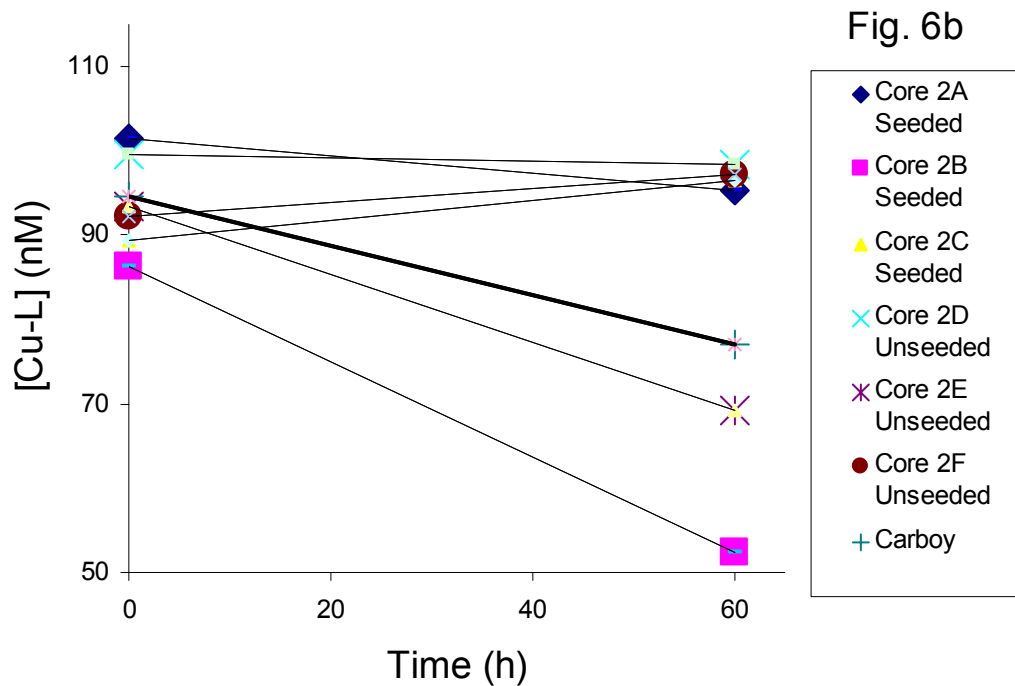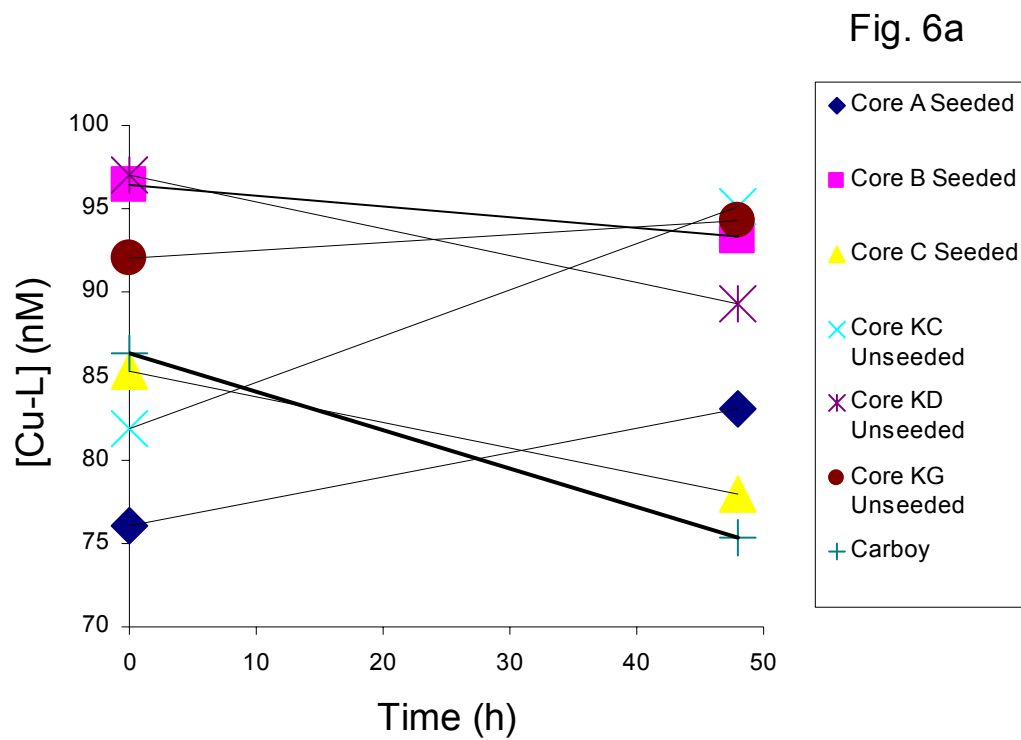- Core 2F Unseeded
- Carboy

Figure 6. Concentration of copper-binding ligands vs. time for six cores during (a) the April 23, 2001 and (b) the June 26, 2001 sediment flux experiments.

Sample	Td [Cu-L] (nM)		Flux
	T = 0	T = 48	(nmol·m^{-2}·d^{-1})
Carboy	86.4 ± 3.3	75.3 ± 2.0	-
Core A seeded	76 ± 0.7	83 ± 7.5	-870
Core B seeded	96.5	93.4 ± 1.1	-400
Core C seeded	85.3 ± 1.4	77.9 ± 0.7	-180
Core KC unseeded	81.9 ± 5.9	95.1 ± 16.4	-1030
Core KD unseeded	97 ± 8.4	89.3 ± 0.8	-160
Core KG unseeded	92 ± 11.4	94.3 ± 1.8	-620

a

Sample	Td [Cu-L] (nM)		Flux
	T = 0	T = 60	(nmol·m^{-2}·d^{-1})
Carboy	94.7 ± 4.1	77 ± 10.7	-
Core 2A seeded	101.5 ± 0.2	95.3 ± 7.5	368
Core 2B seeded	86.4 ± 2.2	52.4 ± 1.2	587
Core 2C seeded	89.4 ± 5.5	96.5 ± 5.4	-813
Core 2D unseeded	99.6 ± 2.4	98.5 ± 2.2	584
Core 2E unseeded	93.3 ± 3.0	69.1 ± 12.7	221
Core 2F unseeded	92.5 ± 3.1	97.3 ± 3.8	-726

b.

Table 8. Copper ligand concentrations and fluxes for the (a) April 23, 2001 experiment and the (b) June 26, 2001 experiment. Negative fluxes denote a net departure of ligands from the sediment to the overlying water. Values are based on the mean of two replicate trials. The reported error is the mean of errors: Error = (|difference in trials |÷ 2).

Fig. 7a

Legend (Fig. 7a):
- Core A (seeded)
- Core B (seeded)
- Core C (seeded)
- Core KC (unseeded)
- Core KD (unseeded)
- Core KG (unseeded)
- Carboy Water (control)

Fig. 7b

Legend (Fig. 7b):
- Core 2A (seeded)
- Core 2B (seeded)
- Core 2C (seeded)
- Core 2D (unseeded)
- Core 2E (unseeded)
- Core 2F (unseeded)
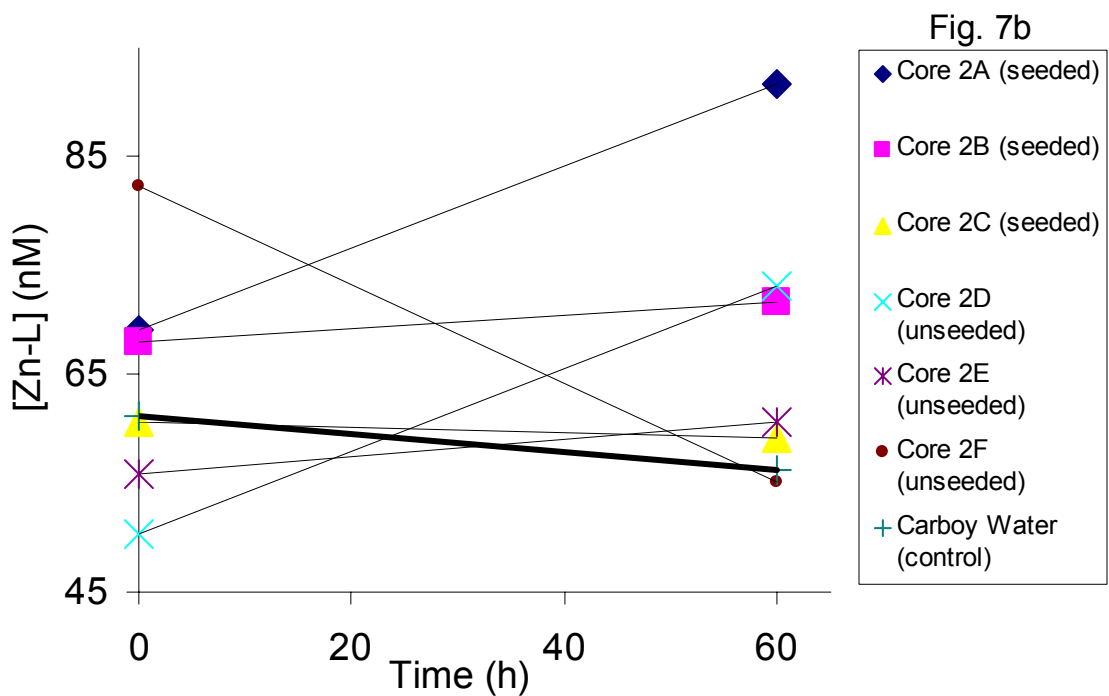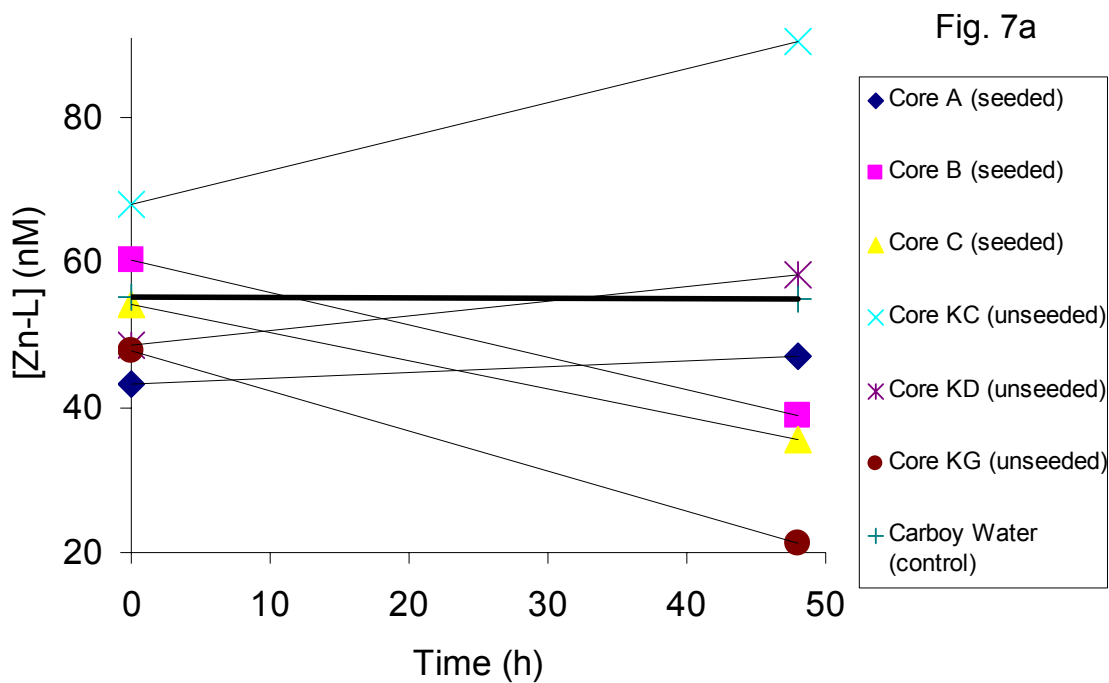- Carboy Water (control)

Figure 7. Concentration of zinc-binding ligands vs. time for six cores during (a) the April 23, 2001 and (b) the June 26, 2001 sediment flux experiments.

Sample	Td [Zn-L] (nM)		Flux
	T = 0	T = 48	(nmol·m^{-2}·d^{-1})
Carboy	55.2 ± 10.0	55.0 ± 13.8	-
Core A seeded	43.3 ± 12.2	47.0 ± 11.2	-187
Core B seeded	60.3 ± 2.6	38.8 ± 14.4	1065
Core C seeded	54.2 ± 25.2	35.6 ± 12.2	911
Core KC unseeded	68.1 ± 33.1	80.4	-531
Core KD unseeded	48.6 ± 1.6	58.2 ± 4.4	-453
Core KG unseeded	47.8 ± 11.7	21.2 ± 1.7	1221

a.

Sample	Td [Zn-L] (nM)		Flux
	T = 0	T = 60	(nmol·m^{-2}·d^{-1})
Carboy	61.2 ± 2.6	56.2 ± 23.9	-
Core 2A seeded	69 ± 10.6	91.8 ± 15.0	-886
Core 2B seeded	68.0 ± 10.1	71.7 ± 0.9	-313
Core 2C seeded	60.6 ± 7.2	59.2 ± 2.6	-115
Core 2D unseeded	50.3 ± 1.7	73.1 ± 12.5	-979
Core 2E unseeded	55.8	60.6 ± 2.0	-333
Core 2F unseeded	82.4 ± 24.7	55.1 ± 2.8	714

b.

Table 9. Zinc ligand concentrations and fluxes for the (a) April 23, 2001 experiment and the (b) June 26, 2001 experiment. Negative fluxes denote a net departure of ligands from the sediment to the overlying water. Values are based on the mean of two replicate trials. The reported error is the mean of errors: Error = (|difference in trials |÷ 2).

fluxes of seeded and unseeded cores. Because this is the first sediment flux experiment to look at the speciation of Zn, there are no other studies of Zn ligand fluxes with which to compare this data.

Because there were no differences noted in the fluxes due to the presence of *S. benedicti*, the six cores in each sampling period could be treated as six replicates. Given this assumption, there is a statistically significant flux of copper-complexing ligands out of the sediment for the April 2001 experiment. However, the two-tailed nonparametric test (Zar, 1984) employed for this analysis is heavily dependent on the standard deviation of fluxes of the samples. In June 2001, for instance, there is a net flux of Cu-binding ligands out of the sediment in 4 of 6 cores, though this does not equate to a statistically significant difference. For Zn-binding ligand concentrations, three of six cores showed a net flux out of the sediment in April 2001, but 5 of 6 cores showed a net flux out of the sediments in June 2001. These last three observations are worth noting for their qualitative significance, as they support the observation that Cape Fear sediments function as a net source of Cu- and Zn-complexing ligands to the overlying water.

The Role of Bioturbation in Fluxes in Cape Fear Estuary

As stated previously, Mann-Whitney tests were used to statistically analyze any differences between the fluxes of the seeded and unseeded cores. There was no significant difference ($\alpha = 0.10$) in the fluxes of any of the species in either sampling period, except for total dissolved Cu on the June 26, 2001 trip. Riedel et al. (1995) found that the presence of macrofauna minimally influenced copper fluxes in oxic sediments but did not attempt to measure the fluxes of any other species included in this study. There are reasons why one might have expected there to be more and larger differences

in fluxes between these two subsets of core samples. First, that *S. benedicti* quickly and dramatically rearranges surface sediments can be observed in the laboratory. Second, pore water studies conducted for various aquatic venues show that sediments at those sites exhibit a concentration gradient of metal-complexing ligands. That is, in these sediments, metal-complexing ligand concentration increases with depth (Skrabal et al., 2000). If the layering of the sediments is disturbed by an organism such as *S. benedicti*, this disturbance could redistribute ligand-rich sediment into the overlying water.

This consequence was not observed, however. This fact could indicate that the concentration gradient in the sediment was very shallow, so that disturbance by *S. benedicti* of the upper sediment did not have a measurable effect. Possibly, the bioturbation by *S. benedicti* is too shallow to have an impact on the fluxes measured in this experiment, and other, deeper-burrowing benthic organisms, such as the common estuarine polychaete, *Mediomastus*, may be important in influencing fluxes.

Characterization of Cu and Zn Ligands

Ruzic plots of the data from the Cu speciation experiments revealed that there is one ligand class that is binding the copper in solution. A plot of Cu^{2+}/Cu-L vs. Cu^{2+} had a good fit to a straight line, which is characteristic of a one-ligand system. Zinc speciation experiments suggest that Zn is bound by two ligand classes. Scatchard plots for most of the Zn speciation titrations gave poor fits to the one-ligand Ruzic model. The shape of these plots, however, is consistent with the graph of a two-ligand system (van den Berg, 1982). The ligands have conditional stability constants very close to one another in value, perhaps as close as 10^8 and 10^{10}, which makes determining the exact concentration and strength of each ligand difficult. A better determination of the stronger

of the two ligand classes might be determined by carrying out titrations with more data points at the low-concentration end of the titration schedule of Zn additions. In order to deal with the mathematically complex task of determining the strengths and concentrations of two different ligand classes present in the same sample, a more complex modeling package, Systat, was employed. Systat uses an iterative algorithm in order to find an equation which converges on the reported data. The Systat analysis was generally unsuccessful because about one fourth of the samples analyzed either did not converge on a solution or did not fit a straight line. However, the samples that did converge suggest rough estimates of the concentration and strengths of the two Zn-binding ligand classes, L_1 and L_2. Based on these data sets that converged, the stronger of the two ligand classes, L_1, has an conditional stability constant K_1 of 10^{10}, and is present in concentrations that range from 6 to 10 nM. The weaker ligand class has a K_2 that ranges from $10^{7.6}$ to 10^8, and is present in concentrations that range from to 20 to 60 nM.

Benthic Fluxes and Cape Fear Estuary

While flux data about metal-binding ligands yields useful information about the biogeochemistry of the estuary, it is also useful to examine how big a contribution a particular flux can make to the standing stock of ligands in an estuarine system. Assuming the residence time of water in the CFR estuary is 7 to 14 days and that the Cu-binding ligand concentration in the overlying water is 50 nM (Shank, 2003), the Cu ligands that enter the water column account for between 0.4% and 4.8% of the total stock of ligands in the water column. This means that the ligands being released from the bottom sediment play a relatively minor role in the total stock of Cu-complexing ligand available. The Zn speciation data follow a similar trend; the contribution that sediment

fluxes make to the standing stock of ligands in the estuary, assuming a overlying water Zn-binding ligand concentration of 50 nM, is between 0.3% and 4.6%. As stated previously, benthic fluxes are not an important source for TDZn (0.2 – 2.6%) or TDCu (0.2-5.6%).

However, it is noteworthy that ligands fluxing from sediments are not distinguishable from those present in the water column, as determined from the analyses of the flux experiments and the bottom water that was used to recharge the cores. This last point is interesting because it suggests that sediments may be a source (albeit a minor one) of Cu- and Zn-complexing ligands found in the water column.

SUMMARY

Copper-binding ligands show statistically significant outward fluxes across the sediment-water interface when the presence of *S.benedicti* was ignored, and a majority of the cores tested showed a net flux of Cu- and Zn- complexing ligands out of the sediment when this same assumption was employed. Seasonality was a significant factor in the flux of total dissolved Cu, which decreased from April to June 2001, but did not affect the fluxes of any of the other chemical species studied. The polychaete, *S. benedicti*, causes rapid rearrangement of the sediment surface in Cape Fear sediments, but did not significantly affect the flux or the nature of any Cu- or Zn-binding ligands at the sites examined in the Cape Fear estuary, nor did it affect the flux of total dissolved Zn. The total dissolved Cu flux was unaffected by the presence of *S. benedicti* in the April experiment, but seeded cores showed a decreased Cu flux for the June experiment. The fluxes of ligands and total dissolved Zn and Cu from the estuarine sediment are not significant contributors to the standing stock of these species in the CFR estuary. However, the ligands that do flux from sediments in the Cape Fear estuary are not distinguishable from those that occur in the overlying water. Thus, a portion of these ligands may have a source in the sediments.

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