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).

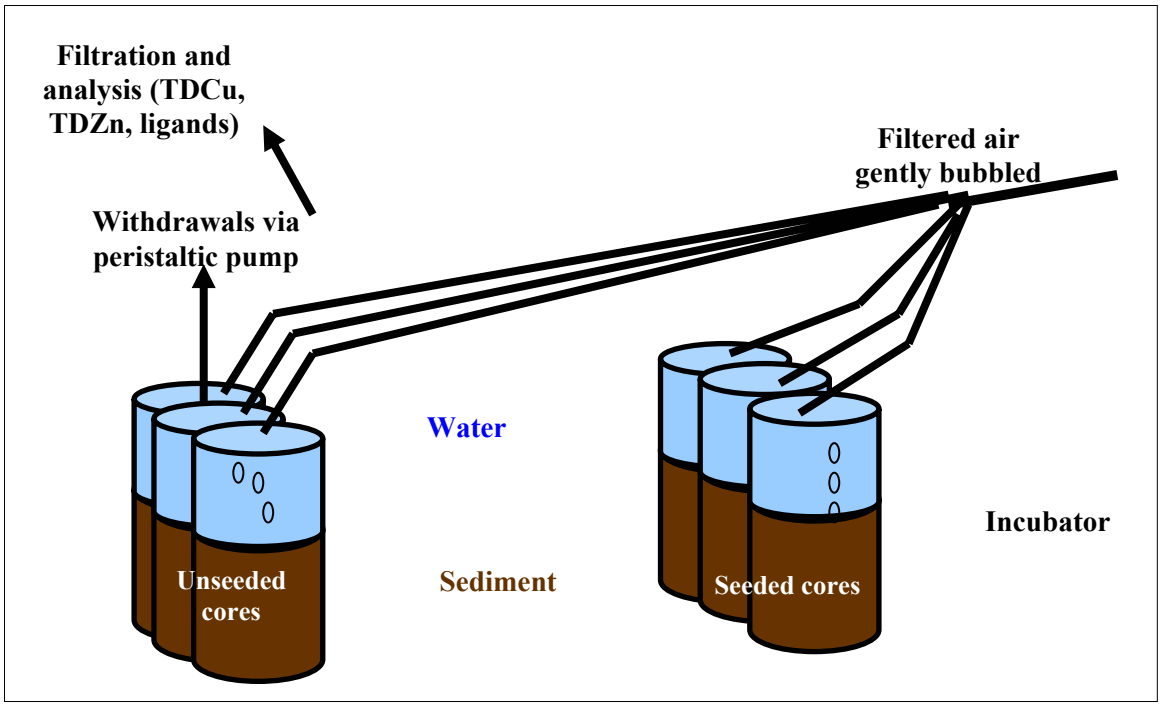

Fig. 3. Diagram of apparatus used in core incubation experiments.

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^{O} 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_4OH	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