

## 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  $\text{nmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  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  $\text{nmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  for the April 2001 experiments to 130 to -24  $\text{nmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  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  $\pm 300 \text{ nmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ . 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

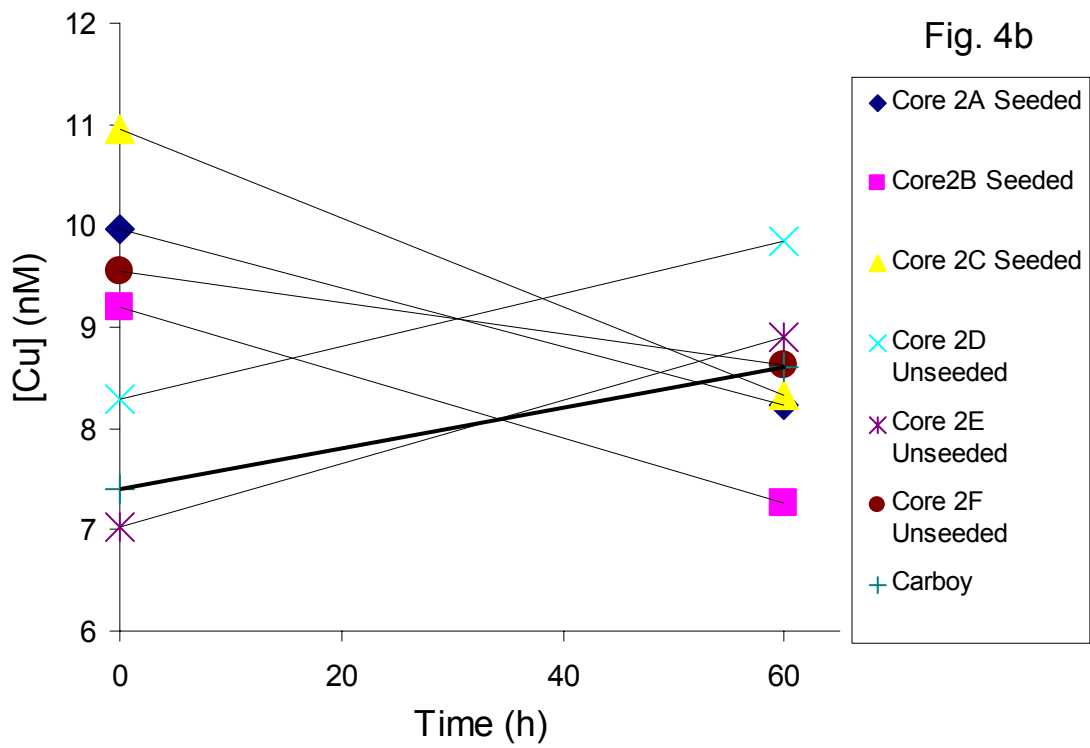
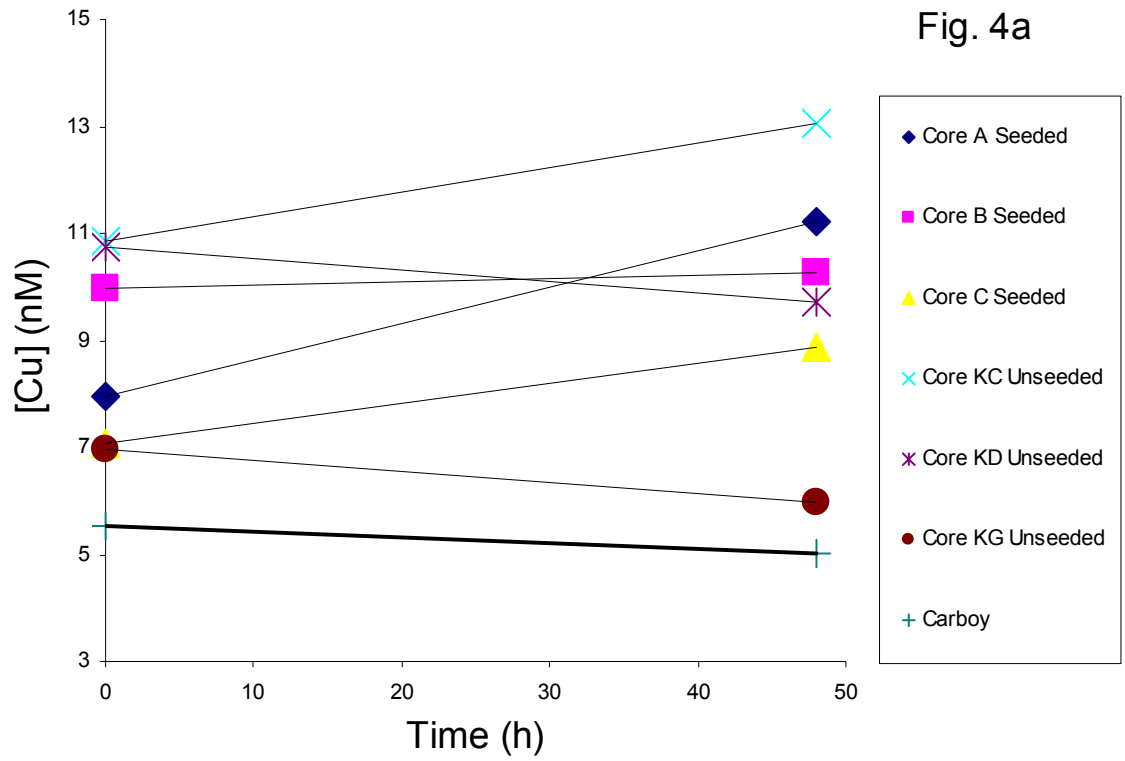


Figure 4. Concentration of total dissolved copper vs. time for six cores during (a) the April 23, 2001 and (b) the June 26, 2001 sediment flux experiments.

Sample	Td [Cu] (nM)		Flux (nmol·m <sup>-2</sup> ·d <sup>-1</sup> )
	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 <sup>-2</sup> ·d <sup>-1</sup> )
	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 <sup>-2</sup> ·d <sup>-1</sup> )		
Location	TDCu	TDZn
Bay of Cadiz <sup>1</sup>	-14 to -411	-
Galveston Bay <sup>2</sup>	-	-2400
Chesapeake Bay <sup>3</sup>	-11 to -85	-
Chesapeake Bay 1994 <sup>4</sup>	-610	-
San Diego Bay 2001 <sup>5</sup>	-1300 to -4200	-1500 to -31,100
Delaware Bay <sup>6</sup>	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  $\text{nmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  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 an 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.

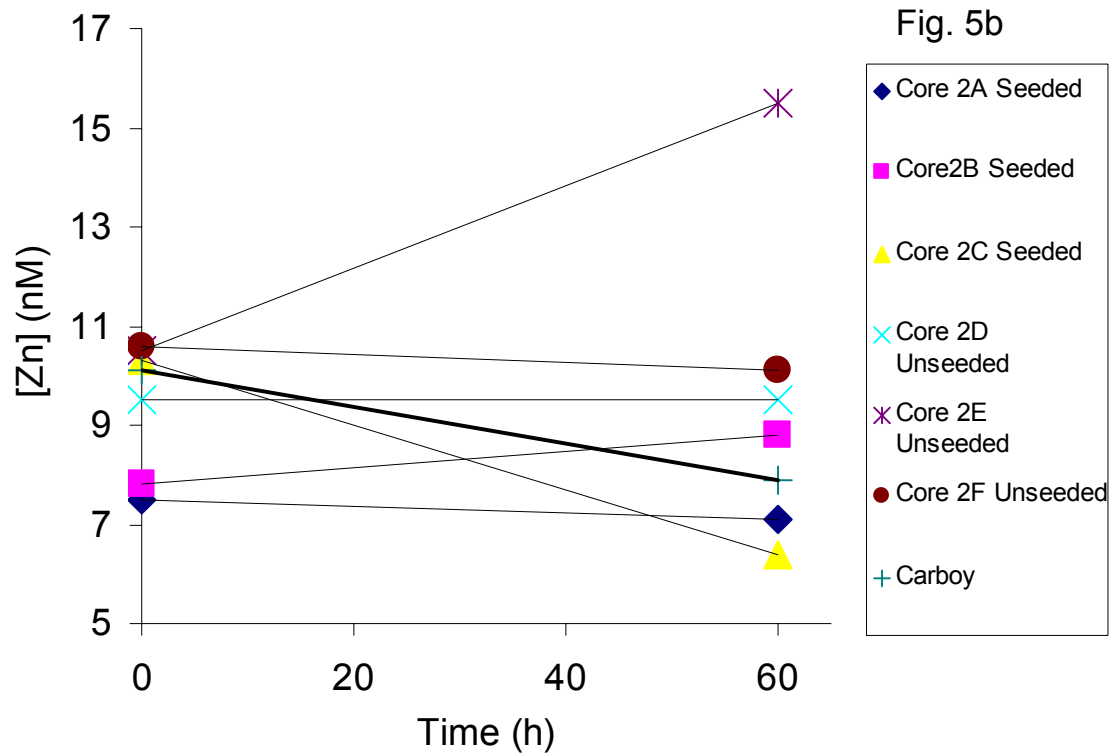
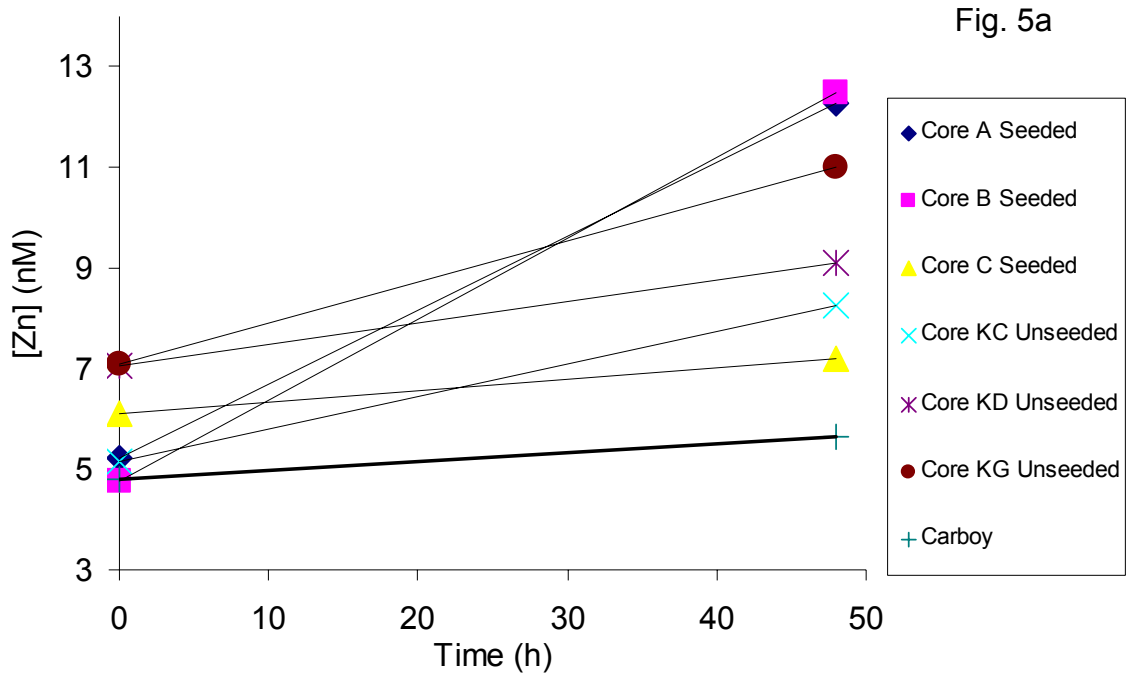


Figure 5. Concentration of total dissolved zinc vs. time for six cores during (5a) the April 23, 2001 and (5b) the June 26, 2001 sediment flux experiments.

Sample	Td [Zn] (nM)		Flux (nmol·m <sup>-2</sup> ·d <sup>-1</sup> )
	T = 0	T = 48	
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 (nmol·m <sup>-2</sup> ·d <sup>-1</sup> )
	T = 0	T = 60	
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 <sup>1</sup>	10	50
Scheldt <sup>2</sup>	10-20	10-50
Sabine <sup>3</sup>	10-15	15-20
SanFrancisco <sub>4</sub>	27-30	6-12
Tamar <sup>5</sup>	40	-
Humber <sup>6</sup>	-	120-150
Ribble <sup>5</sup>	25	-
Galveston Bay <sup>7</sup>	-	10-13
Cape Fear <sup>8</sup>	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  $\text{nmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ . 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  $\text{nmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  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  $\text{nmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ . 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

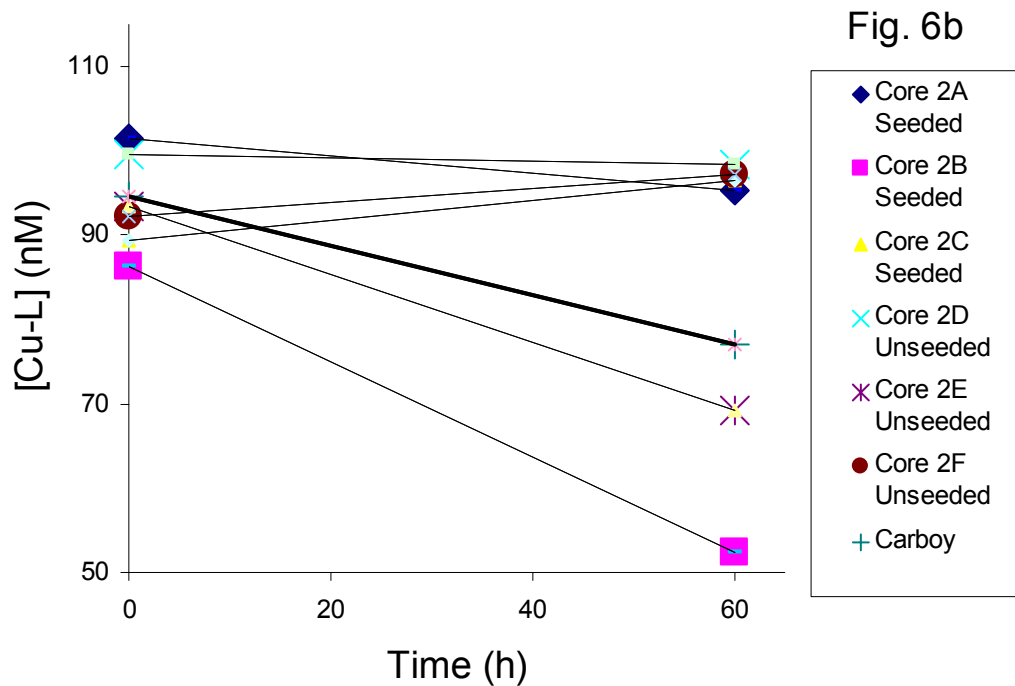
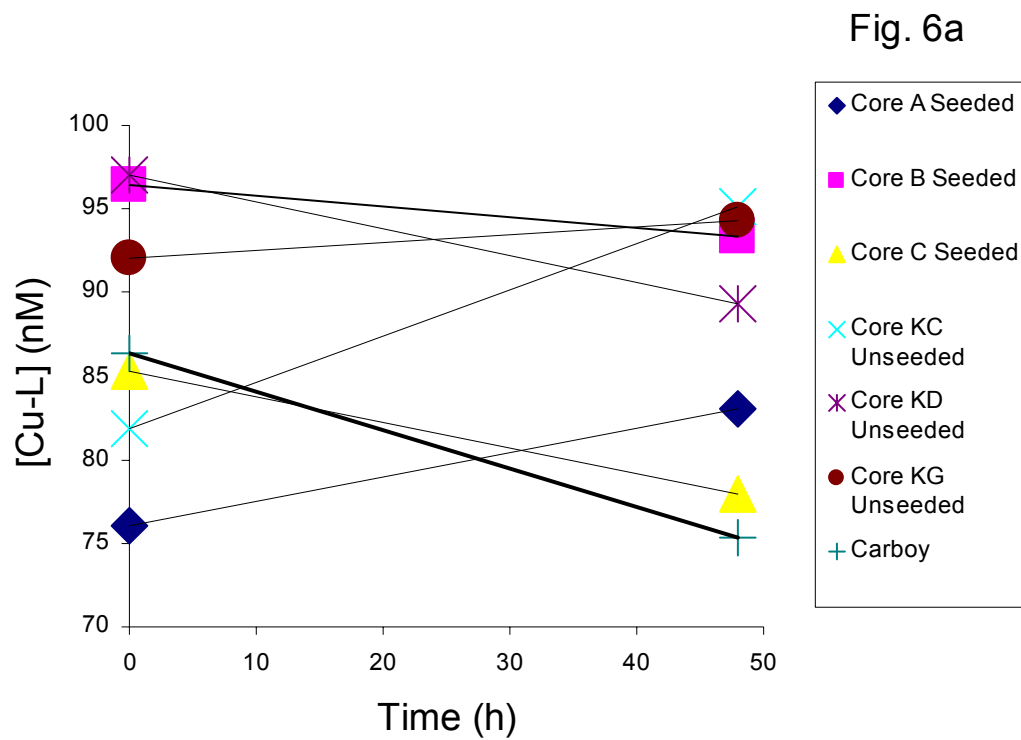


Figure 6. Concentration of copper-binding ligands vs. time for six cores during (a) the April 23, 2001 and (b) the June 26, 2001 sediment flux experiments.

Sample	Td [Cu-L] (nM)		Flux (nmol·m <sup>-2</sup> ·d <sup>-1</sup> )
	T = 0	T = 48	
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 (nmol·m <sup>-2</sup> ·d <sup>-1</sup> )
	T = 0	T = 60	
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).

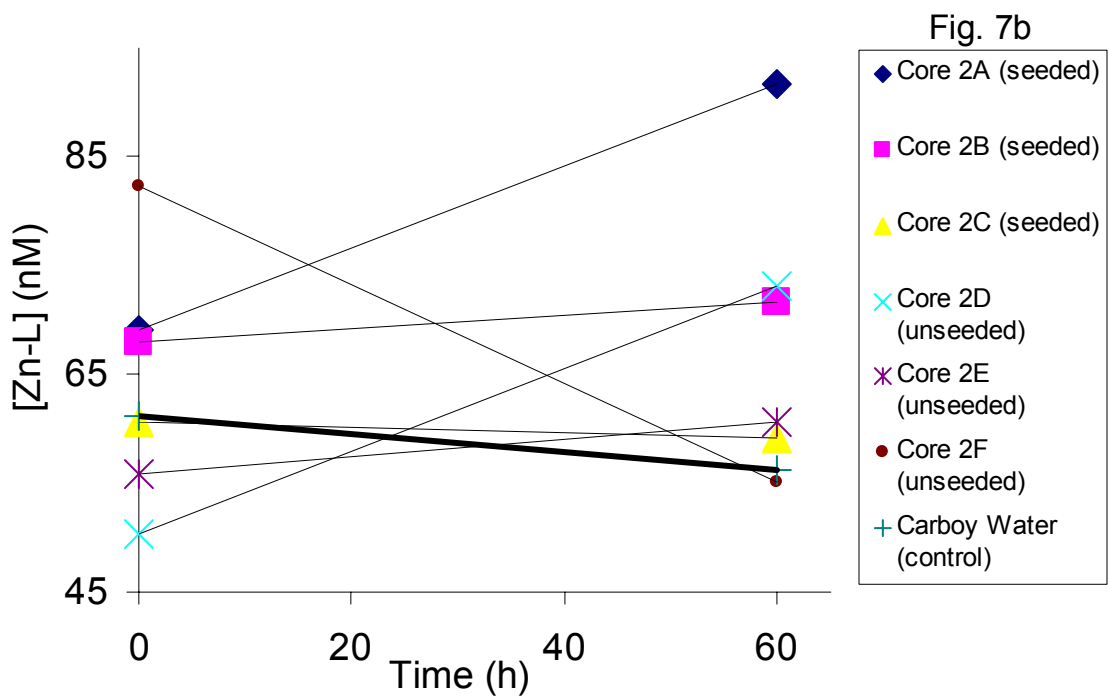
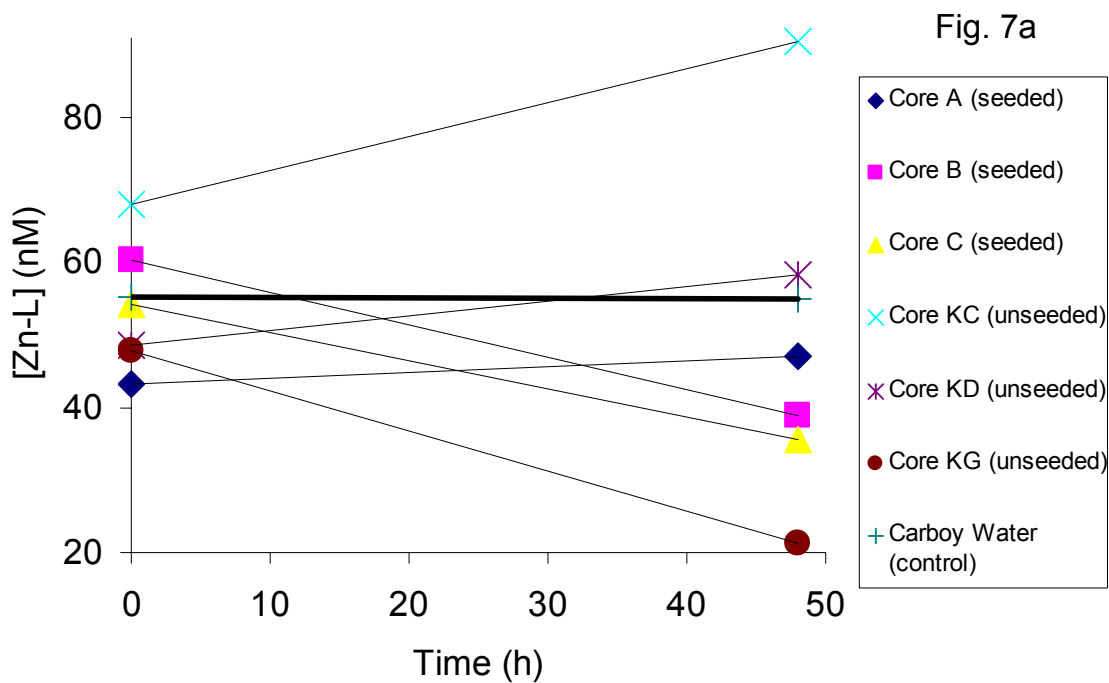


Figure 7. Concentration of zinc-binding ligands vs. time for six cores during (a) the April 23, 2001 and (b) the June 26, 2001 sediment flux experiments.

Sample	Td [Zn-L] (nM)		Flux (nmol·m <sup>-2</sup> ·d <sup>-1</sup> )
	T = 0	T = 48	
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 (nmol·m <sup>-2</sup> ·d <sup>-1</sup> )
	T = 0	T = 60	
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  $\div$  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  $\text{Cu}^{2+}/\text{Cu-L}$  vs.  $\text{Cu}^{2+}$  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.