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## **Copper Complexation Capacity in Surface Waters of the Venice Lagoon.**

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### Abstract

Total copper ( $\text{Cu}_T$ ), copper ion activity ( $\text{pCu}$ ) and the copper complexation capacity ( $\text{CuCC}$ ) were determined in samples of seawater collected in July 2003 from the Venice Lagoon.  $\text{Cu}_T$  and  $\text{CuCC}$  showed considerable spatial variability:  $\text{Cu}_T$  ranged from 1.8 to 69.6 nM, whereas the  $\text{CuCC}$  varied from 195 to 573 nM.  $\text{pCu}$  values varied from 11.6 to 12.6 and are consistent with those previously reported in estuarine and coastal areas (10.9 – 14.1). The range of  $\text{Cu}_T$  values compares well with those reported in the past in the lagoon and in the adjacent Adriatic Sea. The highest concentrations of  $\text{Cu}_T$  were found in samples collected near the industrial area of Porto Marghera, whereas the lowest were measured near the Chioggia and Malamocco inlets, where an intense tidally-driven renewal of seawater takes place. Although  $\text{CuCC}$  showed a high degree of spatial variability, the values recorded in the Venice Lagoon are comparable to those reported in other estuarine systems. In addition,  $\text{CuCC}$  was positively correlated with dissolved organic carbon (DOC), suggesting that organic ligands responsible for Cu complexation are part of the bulk organic matter pool in the lagoon. The  $\text{CuCC}:\text{Cu}_T$  molar ratio was, on average 55:1, suggesting that a large excess of complexation capacity exists in the Venice Lagoon. The high levels of  $\text{CuCC}$  and the narrow range of  $\text{pCu}$  indicates the importance of the role played by organic ligands in controlling the free ion Cu concentrations in the lagoon, and as a consequence, regulating its availability and/or toxicity.

**Keywords:** Copper; Complexation capacity; Copper ion; Venice Lagoon; Adriatic Sea.

## 1. Introduction

In any attempt to describe and quantify the bio-geochemical cycles of trace metals, the physico-chemical form or speciation, is an extremely important characteristic that has to be taken into account (Mueller *et al.*, 2001). It is widely accepted that trace metals can exist in dissolved and/or particulate forms in natural waters; however, much of the uncertainty about the relationship between total metal concentration and toxicity to aquatic organisms results from lack of definitive knowledge of the physico-chemical properties of these metals in natural waters (Donat *et al.*, 1994; Tessier and Turner, 1995).

As the toxicity of metal ions is reported to be far greater than those of its complexes with naturally occurring ligands (Campbell, 1995), complexation of trace metals by organic ligands *in situ*, has been considered the main control on bioavailability and toxicity of trace metals to phytoplankton (Sunda, 1994; Campbell, 1995). It is hypothesized that toxicity to microorganisms decreases because complexation of a metal by organic ligands diminishes its mobility through the cell wall. In addition, the agglomeration and ultimate sedimentation of metal-organic complexes further decreases toxicity by diminishing the concentration of metal ion in the bulk solution.

Previous studies of metal the complexation capacity in seawater have focused on the measurement of the complexation capacity of copper (CuCC) because Cu is toxic to microorganisms even at low levels and is able to form stable complexes with organic ligands (van Veen *et al.*, 2002). Most researchers have reported that more than 80% (generally 99%) of dissolved copper in surface waters is organically complexed (Coale and Bruland, 1988; Donat *et al.*, 1994; Baeyens *et al.*, 1998, Zirino *et al.*, 1998a). Moffett *et al.* (1997) also demonstrated that Cu-binding ligands can be released by cyanobacteria into seawater and, when the sequestering capacity of these ligands is exceeded, Cu can exert acute toxic effects on cyanobacteria above a free copper ion level of  $10^{-11}$  M (Mann *et al.*, 2002). In coastal areas, high concentrations of CuCC generally reduce metal toxicity; therefore, our ability to monitor and regulate copper as a toxin depends on our understanding of the spatial and temporal distributions as well as the sources and sinks of the Cu-binding ligands (e.g. Kogut and Voelker, 2001). Thus, the aim of this study was to determine the extent and spatial variability of CuCC in the Venice Lagoon, an area strongly influenced by human activities.

## 2. Material and Methods.

### 2.1 Study Area.

The Venice Lagoon is a large, semi enclosed water basin (~550 km<sup>2</sup>) bordering the Northern Adriatic Sea (Figure 1). It is approximately 1 m in depth and it includes canals, marshes, and islands. This transition ecosystem is connected to the Adriatic Sea by three inlets, namely Lido, Malamocco and Chioggia. Water exchange with the Adriatic Sea is governed by a semidiurnal tidal regime (Gacic *et al.*, 2004) ranging from 35 cm during neap tides to 100 cm during spring tides, which combined with the shallowness of the lagoon results in large areas of the lagoon are alternately submerged and exposed daily. An underlying network of navigation channels connects the inlets with the main industrial zone of Porto Marghera and the city of Venice, which is situated in the north-central part of the lagoon. Hydrodynamic residence times vary from 1-3 days near the inlets to 20-30 days in the most interior northerly and southerly portions of the lagoon (Cucco and Umgiesser, 2006). Because in the last 500 years, the major rivers that emptied into the lagoon have been diverted, fresh water inflow is small (average =  $3 \times 10^6 \text{ m}^3 \text{ d}^{-1}$ ; Zuliani *et al.*, 2005), less than 1 percent of the flow coming from the Adriatic Sea through the inlets (average =  $6.2 - 9.0 \times 10^8 \text{ m}^3 \text{ d}^{-1}$ ; Gacic *et al.*, 2004; Cucco and Umgiesser, 2006). Hence, the Venice Lagoon differs from other typical estuaries in that it receives very limited freshwater input. In last 15 years, the lagoon has been recovering from heavy anthropogenic activities and uncontrolled discharges of pollutants from both diffuse and point sources, which increased greatly during the post WW II period following urban, industrial, and agricultural development. For example, the direct release of organic micropollutants (Belluci *et al.*, 2000), heavy metals (Bloom *et al.*, 2004; Carrer and Leardi, 2006) and nutrients (Socal *et al.*, 1999) from the industrial area of Porto Marghera is well documented. Recent work done in the lagoon has pointed out that the Venice Lagoon is a mesotrophic environment with an estimated 85 percent of its nutrient nitrogen coming from the agricultural basin surrounding it (Collavini *et al.*, 2005; Zirino, 2005). Its waters have a relatively large amount of nitrogen and ammonia all year-round (Pastres *et al.*, 2004; Acri *et al.*, 2004) and therefore, a relatively high rate of primary production (Martin *et al.*, 1995).

## 2.2. Experimental

### 2.2.1 Sample Collection

The “Magistrato alle acque di Venezia” and “Consorzio Venezia Nuova” set up a water quality monitoring in order to gain a better understanding of the Venice Lagoon (Bendoricchio and De Boni, 2005). Briefly, the MELa1 (Monitoraggio dell'Ecosistema Lagunare) program was carried out for more than 3 years (September 2000 – October 2003) and it included the monitoring of temperature, salinity, redox, plus approximately 20 nutrient-related macrovariables, and 8 trace variables (seven metals + As) once a month in a network of 30 stations covering the whole lagoon (Figure 1). Stations labeled B (for bassifondi; 20) are located in shallow areas; those labeled C (8) are representative of channels, whereas M1 and M2 are located just outside of the inlets of Lido and Chioggia, respectively. A detailed description of major objectives and the experimental design used to carry out MELa1 program can be seen in Solidoro *et al.* (2004a) and Zirino (2005). Thirty samples of surface seawater from the Venice Lagoon were collected in July 2003 at the regularly monitored, 30 MELa1 stations. Briefly, at each station, samples of seawater were collected by using a 5L Niskin sampling bottle; subsamples to be measured for  $\text{Cu}_T$  and  $\text{Cu}_{CC}$  were placed directly in previously acid-washed polyethylene bottles, quick-frozen and shipped under dry ice to the Scripps Institution of Oceanography, where they were maintained frozen until analysis.

### 2.2.2 Reagents and cleaning of materials.

The water used for reagent preparation and rinsing was deionised water (resistivity  $\geq 18.2 \text{ M}\Omega \text{ cm}^{-1}$ ) obtained from a MilliQ<sup>TM</sup> system. Reagents were Ultrex<sup>TM</sup>-grade unless indicated otherwise. Cu standard solution used in the voltammetric and potentiometric determinations was prepared by dissolving analytical grade  $\text{CuSO}_4$  in Milli-Q<sup>TM</sup> water, diluted to give the desired Cu concentration and acidified to pH ~2.0 using 6M HCl. All materials were acid-cleaned in both solutions of HCl and  $\text{HNO}_3$  (10% v/v). All sample manipulations were carried out under a class-100 laminar airflow hood to minimize risks of contamination.

## 2.3. Copper Activity.

The Orion 94-29 copper ion selective electrode (Cu-ISE) was used to determine the  $\text{Cu}^{++}$  activity directly from the samples. Previously, the Cu-ISE electrode was calibrated against two Cu-activity buffers prepared in filtered seawater (0.45  $\mu\text{m}$ ) following the procedure described by Belli and Zirino (1993). These Cu activity buffers were prepared  $2 \times 10^{-4}$  M in Cu and either  $1 \times 10^{-3}$  M glycine or ethylenediamine in filtered seawater (Zirino *et al.*, 1998b). A specific ion interaction model was used to calculate pCu (i.e.,  $-\log[\text{Cu}^{++}]_{\text{aq}}$ ) at the pH and concentrations of major ions in each seawater buffer and, therefore, the calibrated response of the Cu-ISE is reported as the pCu of the solution (Rivera-Duarte and Zirino, 2004). The range of pCu's covered by this calibration was from 9.4 to 13; however, the response of the electrode to these buffers has been demonstrated to be linear over a wider range (Belli and Zirino, 1993).

A double H-cell was used for all calibrations and sample measurements. The first H-cell contained a standard calomel electrode (SCE) in one arm of the H and a saturated KCl solution in the second arm. The two were connected via a sintered glass frit. A second and narrower, H-cell made from flat-bottomed test tubes, contained "clean" seawater in one arm and the Cu-ISE plus the sample, in the other arm. The two arms were separated by another glass frit. The seawater level in the sample arm was always lower than in the reference arm in order not to contaminate the reference. The Cu-ISE nearly filled all space in the arm and reached the bottom of the arm with its sensing crystal placed just above a very small (2mm x 5 mm) stirring bar. This permitted the use of small samples volumes (c.a. 2 ml). A magnetic stirrer was placed below the arm and rotated at high speeds. The two H-cells were connected to each other via a salt bridge made out of a Tygon<sup>TM</sup> tube filled with KCl-saturated agar. The system was allowed to come to equilibrium over months. When measuring, the seawater sample was allowed to equilibrate for two minutes with the ISE and the electrode potential was then recorded. At the end of each suite of measurements, the slope of the Cu-ISE electrode was determined using the Cu-seawater buffers. The average slope for the 28 calibrations carried out was  $29.3 \pm 1.5$  mV decade<sup>-1</sup>, which agrees well with the expected Nernstian slope of 29.6 mV decade<sup>-1</sup>.

## 2.4. Copper Titrations.

All copper titrations were made on unfiltered samples because we were interested in assessing the total binding capacity of the samples. Copper titrations were conducted in the batch

mode as follows: Briefly, sub samples of 10 mL were pipetted into a series of twelve to sixteen 60 ml FEP Teflon bottles. Incremental amounts of a copper standard solution were added to all but two bottles, giving an approximate concentration range of 0 - 800 nM. The addition of spikes changed the pH of the samples by less than 0.1 units. Samples were allowed to equilibrate overnight. At the beginning of next day, the Cu-ISE was conditioned using clean coastal seawater for half an hour before starting the protocol described above. Then, each sub-sample, in incremental Cu concentrations, was introduced into the measurement arm and the potential of the Cu ISE was recorded. (Samples previously measured were removed from the arm by vacuum aspiration through a glass capillary tube). Every determination of the Cu-CC was generated by measuring at least 12 different subsamples. Potentials were then converted to  $[\text{Cu}^{++}]$  and the ligand saturation was indicated by a change in slope on a plot of  $[\text{Cu}^{++}]$  against  $\text{Cu}_T$ . The least square method was used to calculate the straight lines that best indicated where the CC has been exceeded (van Veen *et al.*, 2001). Two straight lines were fitted to the titration data and the intersection point between them was the determined CuCC (See figure 3). In addition, we did not find that freezing the samples had an effect on the CuCC measurement. Briefly, one liter of natural seawater collected at La Jolla, CA, was divided in four aliquots, two were analyzed for CuCC immediately after collection (average =  $225 \pm 6$  nM) and two were processed one week later after being frozen for 8 days (Average =  $232 \pm 3$  nM). There was no statistical difference between these two means.

## 2.5 Determinations of Total Cu

The initial total Cu concentrations ( $\text{Cu}_T$ ) in unfiltered samples were determined by differential pulse anodic stripping voltammetry (DPASV). The measurements were carried out using a CV50W polarographic system (BAS, Inc), connected to a C3 stand. The working electrode was a thin mercury film electrode (TMFE), the reference electrode was a Ag/AgCl electrode and a Pt-wire was used as the auxiliary electrode. Briefly, 10 mL samples were aliquoted in a Teflon™ cell and acidified to pH <2 by addition of 200  $\mu\text{L}$  of Ultrex Grade HCl (Scarano *et al.*, 1990). After first purging the sample for 6–8 min with ultrapure nitrogen, the Teflon™ stirring bar was switched on, and the Cu was concentrated on the working electrode for 15 min at a potential of -0.8 V versus the Ag/AgCl electrode. After plating, the stirrer was turned



off and 25 s later, the voltage scan was started in the positive direction in the differential pulse mode. The scan parameters included a scan rate of  $10 \text{ mV s}^{-1}$ , a pulse amplitude of 25 mV and final potential of  $-0.01 \text{ V}$ . The copper oxidation wave was recorded between  $-0.25$  and  $-0.05 \text{ V}$ ; the signal height peak of each voltammogram was processed manually. In order to calculate the total concentration of Cu in each sample, the standard addition method was applied with at least, two additional spikes of Cu added (Figure 2). Usually, the coefficients of correlation ( $r^2$ ) were better than 99%. Replicated analysis of a single sample were different by less than 7% and the accuracy of the method was assessed by analyzing a certified reference material (CASS3) from the National Research Council of Canada and values obtained were within certified 95% confidence intervals.

### 3. Results and discussion

The validity of pCu and CuCC determinations in seawater using the Orion 94-29 Cu-ISE has been established previously (Zirino *et al.*, 1998b; Eriksen *et al.*, 1999; Rivera-Duarte and Zirino, 2004). Eriksen *et al.* (2001) have also shown that the Cu ISE provide a reproducible measure of the toxicity of Cu in cultures of marine phytoplankton (*Nitzschia closterium* and *Dunaliella tertiolecta*), whereas Rivera-Duarte *et al.* (2005) showed that the equivalence point determined with the Cu-ISE coincided with the results obtained using U.S. EPA-approved bioassay methods. In this study, the determined pCu values varied from 11.6 to 12.6 (Table I), and are consistent with those previously reported in estuarine and coastal areas (10.9 – 14.1; Rivera-Duarte *et al.*, 2005 and references therein). Thus, the free ion concentrations of Cu recorded in the Venice Lagoon were lower than  $10^{-10} \text{ M}$ , which are below the concentrations of toxic responses reported from laboratory experiments with *Monochrysis lutheri* (Sunda and Lewis, 1978), *Gonyaulax tamarensis* (Anderson and Morel, 1978), several marine diatoms as *Asterionella glacialis* and *Bacteriastrum delicatulum* (Brand *et al.*, 1986) and, the cyanobacteria *Synechococcus spp* (Moffett and Brand, 1996; Moffett *et al.*, 1997, Croot *et al.*, 2003) and *Prochlorococcus spp* (Mann *et al.*, 2002).

The ligand concentrations in natural waters are often quantified by various titration methods (Buffle, 1990). The Cu-ISE Cu titrations conducted on water samples from the Venice Lagoon behaved as typical titration curves with a higher slope with increasing Cu concentration

(Figure 3). All samples showed weak curvature at the lower added Cu concentrations, thus indicating the presence of natural Cu binding ligands. Although the presence and the exact value of the constant of stability of the different type of ligands present in the samples cannot be accurately described with our methodology, these metal titrations provide relevant information about the total metal buffering capacity within the range of the copper additions used. Thus, only ligand concentrations data will be further discussed in the present work.

### ***3.1 Spatial distribution of $Cu_T$ and $Cu_{CC}$ in the Venice Lagoon.***

The average  $Cu_T$  in this study was  $15.4 \pm 3.0$  nM, ranging between 1.8 nM measured at the southern area of the lagoon to 69.6 nM recorded off the industrial area of Porto Marghera (Table I). These values compare well with those reported previously by Martin *et al.* (1994; 2000), who collected several samples in the north part of the lagoon in two surveys carried out in 1992 under spring (average Cu =  $14.4 \pm 4.4$  nM) and winter (average Cu =  $8.1 \pm 2.3$  nM) conditions. Our data set also compares favorably with those reported for surface waters of the northern Adriatic Sea (Tankere and Statham, 1996; Zago *et al.*, 2002), which were measured under winter and summer conditions (average Cu =  $7.7 \pm 3$  nM).

The lower  $Cu_T$  concentrations were found close to the Malamocco (average =  $6.4 \pm 3.1$  nM; n = 2) and Chioggia ( $6.1 \pm 2.6$  nM; n = 2) inlets, which are subject to an intense tidal water renewal (Gacic *et al.*, 2004; Solidoro *et al.*, 2004b; Cucco and Umgiesser, 2006). In contrast, at the Lido inlet, the average  $Cu_T$  was 22.1 nM (n = 2; Table I). The highest  $Cu_T$  was 69.6 nM measured at station 8B located off Porto Marghera, which is the industrial area. This high concentration of Cu may be linked to the disposal of industrial wastes and the fact that this area is poorly flushed by tides and freshwater inputs from Mestre-Marghera (Sfriso *et al.*, 1995; Carrer and Leardi, 2006). Our findings are consistent with previous reports, which have documented the direct release of organic micropollutants (Belluci *et al.*, 2000) and of heavy metals (Sfriso *et al.*, 1995; Bloom *et al.*, 2004) from the industrial zone of Porto Marghera. For instance, Bernardello *et al.* (2006) recently measured the Cu concentration in sediments, and they found a clear gradient decreasing from Porto Marghera toward the sea inlets, suggesting the primary role of the industrial zone as a source of heavy metals to the Venice Lagoon.

The CuCC ranged from 195 nM at Malamocco inlet to 573 nM recorded at station 20B in the southern lagoon (Table I). These CuCC values are comparable to those reported in other coastal lagoons and estuarine systems. For example, values of CuCC ranging between 98 and 338 nM have been reported in San Diego Bay (Rivera-Duarte *et al.*, 2005). van Veen *et al.* (2001) found maximum values between 200 and 420 nM in the lower salinity samples collected at the Humber estuary. Similarly, in the Venetian lagoon, CuCC values between 358 and 556 nM were found in waters with salinities lower than 32 (Table I).

Based on the similarity of the temporal evolution of nutrients and chlorophyll-a (Chl-*a*) collected during 2001 in the same MELa1 sampling grid, Solidoro *et al.* (2004a) grouped the stations in the lagoon into several clusters. Among them were “marine” stations, physically located closest to the Adriatic Sea, “estuarine” stations, situated close to the tributary discharges, and stations placed in the southern part of the lagoon. Accordingly to the Solidoro *et al.* (2004a) classification, the “marine” samples had values of CuCC ranging between 195 and 312 nM (average =  $267 \pm 40$  nM;  $n = 6$ ) and were statistically lower ( $t_{\text{student}}$ ;  $p < 0.05$ ) than those found south of Malamocco inlet in the southern area of the lagoon (average =  $500 \pm 40$  nM;  $n = 6$ ) and lower than those from the estuarine region (average =  $439 \pm 62$  nM;  $n = 4$ ) of the lagoon, where salinity was lower than 32. In particular, CuCC values as high as 520 nM were measured in the brackish area ( $S < 32$ ) close to the mouth of Sile River. These results could suggest that Cu-binding ligands may be produced in the estuarine area inside the lagoon. Moreover, these findings also suggest that the Venice Lagoon is functioning as a source of Cu-binding ligands to the Adriatic Sea (see further discussion below).

### **3.2 Relationship between $Cu_T$ and CuCC.**

In this study, the CuCC was not correlated with  $Cu_T$  as reported in other coastal systems. For example, in Newport Bay, RI, the concentrations of Cu complexation ligands increased with increasing total Cu concentrations (Kozelka and Bruland, 1998). Likewise, in Galveston Bay, which is a shallow lagoon much more similar to the Venice Lagoon, Tang *et al.* (2002) found high CuCC associated with  $Cu_T$ . However, in the Venice Lagoon, the CuCC: $Cu_T$  molar ratio was much higher, 55:1 on average, indicating that a large excess complexation capacity exists and because of this excess of CuCC, almost all Cu is likely to be organically complexed. Similar

results have been reported for the adjacent Adriatic Sea (Zago *et al.*, 2002) and the eastern coast of USA (Shank *et al.*, 2004a). For example, in Cape Fear River, strong Cu-complexing ligands concentrations ranging from 7 to >200 nM were also found in excess of dissolved Cu concentrations (3 - 25 nM; Shank *et al.*, 2004a). These results suggests that the ecological importance of the CuCC may go beyond Cu, since the excess of ligands detected with  $\text{Cu}^{++}$  does not necessarily mean that the organic ligands are specific to Cu but may also bind other trace metals.

### 3.3 Relationship between DOC and salinity.

The average DOC concentration in this study was  $308 \pm 35 \mu\text{M}$ , ranging between  $99.5 \mu\text{M}$ , measured at station 1C located in the northern area, and  $766 \mu\text{M}$  recorded at station 20B in the southern part of the lagoon (Table I). In addition, higher values of DOC ( $339 \pm 90 \mu\text{M}$ ) were generally recorded within the estuarine part of the lagoon whereas the lower were found in stations near to the inlets ( $201 \pm 36 \mu\text{M}$ ). These DOC concentrations compares favorably with those reported previously by Martin *et al.* (1995), who measured DOC concentrations between 116 and  $312 \mu\text{M}$  along a transect from the Silone channel to the Lido inlet. However, it is worth noting that DOC concentrations measured in the Venice Lagoon are higher than other Mediterranean coastal regions (Martin *et al.*, 1995) and the adjacent northern Adriatic Sea (Pettine *et al.*, 1999; De Vittor *et al.*, 2008). The difference in DOC concentration between the lagoon and the Adriatic Sea also indicates that the Venice Lagoon may be a locally important source of organic matter to the adjacent Adriatic Sea.

In order to understand better the DOC distribution in the lagoon, we used the MELa1 data set for the period from September 2000 to October 2003 ( $n = 1170$ ; 30 stations sampled in 39 campaigns). The distribution of DOC versus salinity exhibited an inverse relationship, with lower values in the marine end member and higher in the estuarine part of the lagoon (Figure 4a). River inflow probably does not account for these high DOC concentrations found in the estuarine area of the Venice lagoon, because in the past the main freshwater tributaries were diverted out of the lagoon and it receives very limited freshwater input. Compared with the flow exchanged with the Adriatic Sea through the inlets (Gacic *et al.*, 2004), the freshwater input is less than 1 percent (Zuliani *et al.*, 2005). An estimate of the increase of DOC in surface water of the Venice

Lagoon as a result of riverine input, can be made by taking the mean DOC riverine concentration, the mean flux of freshwater (average =  $3 \times 10^6 \text{ m}^3 \text{ d}^{-1}$ ; Zuliani *et al.*, 2005), an average hydrodynamic residence time of 15 days (Cucco and Umgiesser, 2006) and, the mean depth of the lagoon (~1.0 m). Although DOC data reported for the riverine inputs arriving to the Venice Lagoon are scarce, an average of  $296 \pm 40 \text{ } \mu\text{M}$  can be calculated from those reported for the Silone ( $179 \pm 57 \text{ } \mu\text{M}$ ; Martin *et al.*, 1995; Dai *et al.*, 2000) and Osellino ( $416 \text{ } \mu\text{M}$ ; Critto *et al.*, 2004) channels. Thus, the DOC concentration in the surface water of the Venice Lagoon would have an average increase of approximately  $24 \text{ } \mu\text{M}$  in 15 days ( $1.6 \text{ } \mu\text{M d}^{-1}$ ), or an increase of only 11% of the mean DOC concentration ( $223 \pm 45 \text{ } \mu\text{M}$ ) measured during all MELa1 campaigns, suggesting that the riverine input of DOC is not so important for the surface waters of the lagoon.

The mixing diagram also showed that DOC distributions deviated positively from conservative mixing (Figure 4a), indicating production of these constituents within the lagoon. Similarly, Martin *et al.*, (1995) reported a non conservative behavior of DOC within the northern part of the Venice Lagoon. Thus, these findings indicate that the origin of organic matter in the Venice Lagoon is mainly autochthonous. Moreover, for the same MELa1 data set for the period from September 2000 to October 2003, we found high values of DOC associated with high chlorophyll concentrations ( $r = 0.80$ ;  $p < 0.05$ ;  $n = 30$  stations; Figure 4c), substantiating the idea that DOC is partially produced by phytoplankton primary producers within the lagoon (Figure 4b). The high primary production of the lagoon largely determines the distribution of DOC and Chl *a*, leading to spatial distribution patterns with maxima at intermediate salinities rather than at the river end member as in other estuaries (Figure 4a, b). Hence, primary production in the Venice Lagoon probably plays an important role as a source of organic matter and as a consequence, of CuCC.

### **3.4 Relationship between DOC and CuCC.**

Although concentrations of Cu complexing ligands measured in this study were only a small fraction (0.1 - 0.5%) of the total water column DOC concentrations (Table I), figure 5 shows that CuCC varied in proportion to changes in DOC concentrations ( $r = 0.60$ ,  $n = 25$ ,  $p < 0.05$ ). As DOC in the Venice lagoon is, on average, 82% of the total organic

carbon, it is very likely that the ligands responsible for Cu complexation are part of the bulk organic matter pool. Our results are consistent with other studies, which have also shown a positive relationship between Cu complexation and bulk organic matter concentrations (Gerringa *et al.*, 1998; Shank *et al.*, 2004a, b). For instance, in the organic-rich Cape Fear estuary (USA), DOC and ligand concentrations exhibited strong linear correlations ( $r^2 = 0.93$ ,  $p < 0.01$ ) among transect samples at DOC concentrations  $< 1000 \mu\text{M}$  and among all size fractions of ultrafiltered estuarine samples ( $r^2 = 0.94$ ; Shank *et al.*, 2004a). Similarly, Tang *et al.* (2001) have demonstrated the importance of colloidal ligands in the organic complexation of Cu in brackish waters of Galveston Bay (USA) and Zirino *et al.* (1998a) reached the same conclusions in San Diego Bay. In the Venice Lagoon, Dai *et al.* (2000) reported that a significant part (46%) of the measured dissolved Cu was actually associated with the organic colloidal material. Thus, colloidal organic carbon may be the major vehicle in the formation of organic-metal complexes in the water column of estuarine systems (Mackey and Zirino, 1994).

Although it has been known for some time that organic ligands control Cu speciation in most natural waters, the sources of these ligands are not fully understood. In estuarine environments, ligands may derive from anthropogenic and terrestrial sources (Shank *et al.*, 2004a, b), sediment (Skrabal *et al.*, 1997), and from water column processes (Dryden *et al.*, 2004). For example, Shank *et al.* (2004b) showed that an important source of CuCC complexing ligands to the waters in the South Atlantic Bight is provided by the discharge of dissolved organic matter (DOM) by the rivers along the coastal North Carolina (USA). This is not the case for the Venice Lagoon because the organic matter content (Critto *et al.*, 200; Dai *et al.*, 2000) and the freshwater input (Zuliani *et al.*, 2005) are very much reduced.

Sediments are also likely to play an important role as a source of organic matter to the water column in the Venice Lagoon. The prevalence of a semidiurnal tidal regime combined with an average water depth of 1m, generates vigorous currents which are effective in mixing the water column, and at the same time, provokes that large areas in the lagoon are alternately submerged and exposed on a daily basis (Cucco and Umgiesser, 2006). Consequently, Cu-complexing ligands released from sediment pore waters may also influence Cu speciation. Five samples of porewater collected at 5 different locations in the lagoon resulted in range of CuCC values of 315 to 676 (average =  $506 \pm 141 \text{ nM}$ ). These CuCC levels in porewater from the

Venice Lagoon fall in the range reported for porewater from Chesapeake Bay (135 - 12500 nM; Skrabal *et al.*, 2000). Although our data set is very limited for the number of samples analyzed, it shows high variability and suggests that sediments could be a potentially important source of CuCC to the overlying water column in the Venice Lagoon. Other researchers have previously demonstrated that bottom sediments are an important source of CuCC in coastal systems as Chesapeake Bay (Skrabal *et al.*, 1997; 2000) and Cape Fear River Estuary (Shank *et al.*, 2004c) in USA. In Chesapeake Bay, for example, the measured benthic fluxes of CuCC ranged between 300 and 1200 nmol m<sup>-2</sup> d<sup>-1</sup>, suggesting that benthic fluxes may supply from 10 to 50% of the standing stock of CuCC in this bay (Skrabal *et al.*, 1997). Finally, it is important to note the important difference in electrochemical methodologies used in these studies. Skrabal *et al.* (1997; 2000) and Shank *et al.* (2004c) were examining certain ligand types that may or may not be the same as the CuCC measured in this study carried out in the Venice Lagoon.

The high variability of CuCC in the Venice Lagoon can be explained partially because there is a superimposition of the labile DOM released by biological activity and the pool of more resistant refractory organic material. Both fractions of organic matter may become associated with Cu with different efficiencies of complexation. The more labile organic material is found in productive surface waters released by the recent biological activity of some species of phytoplankton (Brand *et al.*, 1986; Moffet *et al.*, 1997), macroalgae (Gledhill *et al.*, 1999), bacterioplankton (Dryden *et al.*, 2004) and heterotrophic bacteria (Gordon *et al.*, 2000), all of which have the potential to contribute to the pool of strong Cu complexing ligands in natural waters. In the past, the Venice Lagoon was heavily influenced by the massive proliferation of the macroalgae *Ulva rigida*. Now, a remarkable disappearance of this species has been reported (Sfriso and Marcomini, 1996; Sfriso *et al.*, 2003); therefore, other seaweed and phytoplankton communities are now the most important primary producers in the lagoon (Facca *et al.*, 2002). Thus, it is expected that the spatial and temporal variation in CuCC is associated with the occurrence or variability of the different producers of organic matter in the lagoon.

The exact nature and sources of the ligands in the water column of the Venice Lagoon remain largely unknown. The high levels of CuCC and the narrow range of pCu recorded in this study suggest the importance of the organic ligands controlling the free ion Cu concentrations in the Venice Lagoon, and as a consequence, in regulating its availability and/or toxicity. Thus, the

observations and conclusions drawn here may apply to other lagoons characterized by the presence of high organic carbon concentrations. For example, the contribution of additional organic ligands may reduce copper availability and toxicity to primary producers in lagoons subjected to very high organic matter release.

#### 4. Conclusions.

This work focuses on studying the spatial variability of  $\text{Cu}_T$ ,  $\text{pCu}$  and  $\text{CuCC}$  from the Venice Lagoon in July 2003.  $\text{Cu}_T$  and  $\text{CuCC}$  showed considerable spatial variability and the range of  $\text{Cu}_T$  values compares well with those reported in the past in the lagoon and in the adjacent Adriatic Sea.  $\text{pCu}$  values varied from 11.6 to 12.6 and are consistent with those previously reported in estuarine and coastal areas. The highest concentrations of  $\text{Cu}_T$  were found in samples collected near the industrial area of Porto Marghera, whereas the lowest were measured near the Chioggia and Malamocco inlets, where an intense tidally-driven renewal of seawater takes place. Although  $\text{CuCC}$  showed a high degree of spatial variability, the values recorded in the Venice Lagoon are comparable to those reported in other estuarine systems. In addition,  $\text{CuCC}$  measured in this study was positively correlated with DOC, suggesting that organic ligands responsible for Cu complexation are part of the bulk organic matter pool in the lagoon. The  $\text{CuCC}:\text{Cu}_T$  molar ratio was, on average 55:1, suggesting that a large excess of complexation capacity exists in the Venice Lagoon. However, the exact nature and sources of the ligands in the water column of the Venice Lagoon remain largely unknown. Finally, the high levels of  $\text{CuCC}$  and the narrow range of  $\text{pCu}$  suggest the importance of role played by organic ligands controlling the free ion Cu concentrations in the lagoon, and as a consequence, regulating its availability and/or toxicity.



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**Figure captions**

Figure 1.- Study area and location of MELa1 sampling stations in the Venice Lagoon. Symbols indicate hydrographic sampling stations classified as function of salinity according to Solidoro *et al.* (2004a). Marine stations are those with salinity  $>33.0$  ( $\circ$ ), estuarine stations with salinity  $<31.0$  ( $\blacksquare$ ) and intermediate stations with salinities between 31.0 and 33.0 ( $\blacktriangledown$ ). B stands for stations located in shallow areas, whereas those marked as C were positioned at the channels.

Figure 2.- Representative ASV-standard addition curves for the determination of total Cu concentration in acidified ( $\text{pH} < 2$ ) samples collected at stations 19B and 8C from the Venice Lagoon in July 2003.

Figure 3.- Representative titration curves of Cu complexation capacity in samples collected at stations 19B and 8C from the Venice Lagoon in July 2003.

Figure 4.- Relationship between (a) the monthly dissolved organic carbon (DOC) and (b) chlorophyll *a* concentrations and salinity in the Venice Lagoon from September 2000 to October 2003. (c) Relationship between the monthly DOC and chlorophyll *a* concentrations during the same period. Each point represents the average of thirty-nine samplings (2000-2003) of chlorophyll *a* and DOC concentrations at each of the thirty lagoon stations. The brackets indicate the standard error. The station symbols indicate the relative salinities; marine stations are those with salinity  $>33.0$  ( $\circ$ ), estuarine stations with salinity  $<31.0$  ( $\blacksquare$ ), and intermediate stations with salinities between 31.0 and 33.0 ( $\blacktriangledown$ ). The conservative line in (a) was constructed using a simple two end-member mixing model (e.g. Eyre, 2000), based on DOC average values previously reported for the Northern Adriatic Sea ( $151 \pm 40 \mu\text{M}$ ; Pettine *et al.*, 1999) and for the freshwater input arriving to the Venice Lagoon ( $296 \pm 40 \mu\text{M}$ ; Martin *et al.*, 1995; Critto *et al.*, 2005).

Figure 5.- Relationship between CuCC and DOC in the Venice Lagoon. Marine stations are those with salinity  $>33.0$  ( $\circ$ ), estuarine stations with salinity  $<31.0$  ( $\blacksquare$ ) and intermediate stations with salinities between 31.0 and 33.0 ( $\blacktriangledown$ ) according to Solidoro *et al.* (2004a).

Table I.- Salinity, Cu complexation capacity (CuCC), total Cu, pCu, chlorophyll a and dissolved organic carbon (DOC) for samples collected in the Venice Lagoon during July 2003.

$$\text{pCu} = -\log[\text{Cu}^{++}]_{\text{aq}}.$$

Sample #	Station	Salinity	CuCC (nM)	total Cu (nM)	pCu	Chl a (µg/L)	DOC (µM)
1	13B	36.75	195	9.6	12.04	1.4	---
2	4B	33.02	266	20.6	11.56	16.0	174.7
3	2C	28.11	282	17.0	12.08	21.0	283.1
4	3C	35.09	266	12.9	12.20	14.0	141.4
5	1M	36.48	312	22.1	12.23	2.2	158.0
6	2M	36.84	257	4.3	11.64	2.2	174.7
7	8B	31.18	400	69.6	11.98	43.0	307.9
8	20B	34.47	573	60.9	12.13	3.6	766.1
9	5C	35.20	446	23.5	11.81	7.7	182.9
10	4C	36.28	279	22.1	11.53	1.0	224.7
11	12B	36.20	274	4.2	11.69	1.4	124.7
12	8C	36.37	288	27.1	12.05	1.3	249.7
13	14B	36.96	473	3.4	11.38	1.9	199.5
14	10B	31.03	556	25.9	11.79	15.0	474.4
15	3B	37.15	368	4.0	11.81	4.3	166.3
16	2B	32.71	358	---	12.07	12.0	291.3
17	19B	36.83	484	8.0	11.76	1.5	341.2
18	7C	37.03	272	6.2	11.99	1.7	---
19	5B	33.06	523	3.9	11.96	9.2	224.5
20	17B	36.28	---	3.7	12.20	2.3	---
21	6C	35.34	517	5.5	12.17	5.8	216.1
22	1C	36.61	451	7.4	11.89	2.1	99.5
23	6B	33.43	---	7.0	12.09	8.1	---
24	15B	36.32	464	1.8	12.52	3.3	441.2
25	11B	36.06	497	12.8	12.62	2.7	657.8
26	16B	34.64	514	6.1	12.19	3.3	324.5
27	18B	36.58	495	7.1	12.36	1.3	616.2
28	1B	27.68	516	5.5	12.23	32.0	---
29	7B	33.71	407	22.3	12.48	11.0	474.6
30	9B	34.01	503	21.8	12.19	11.0	382.8

Table I.

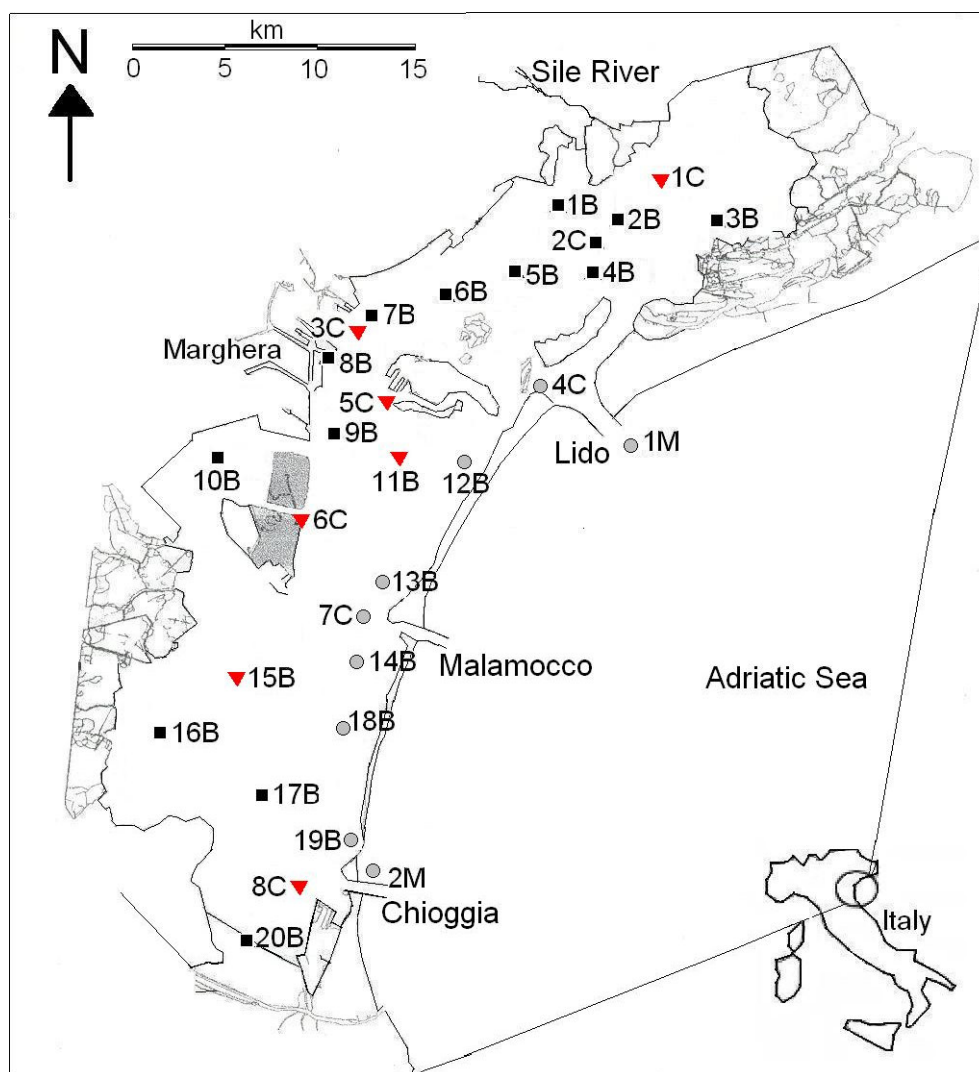


Figure 1.



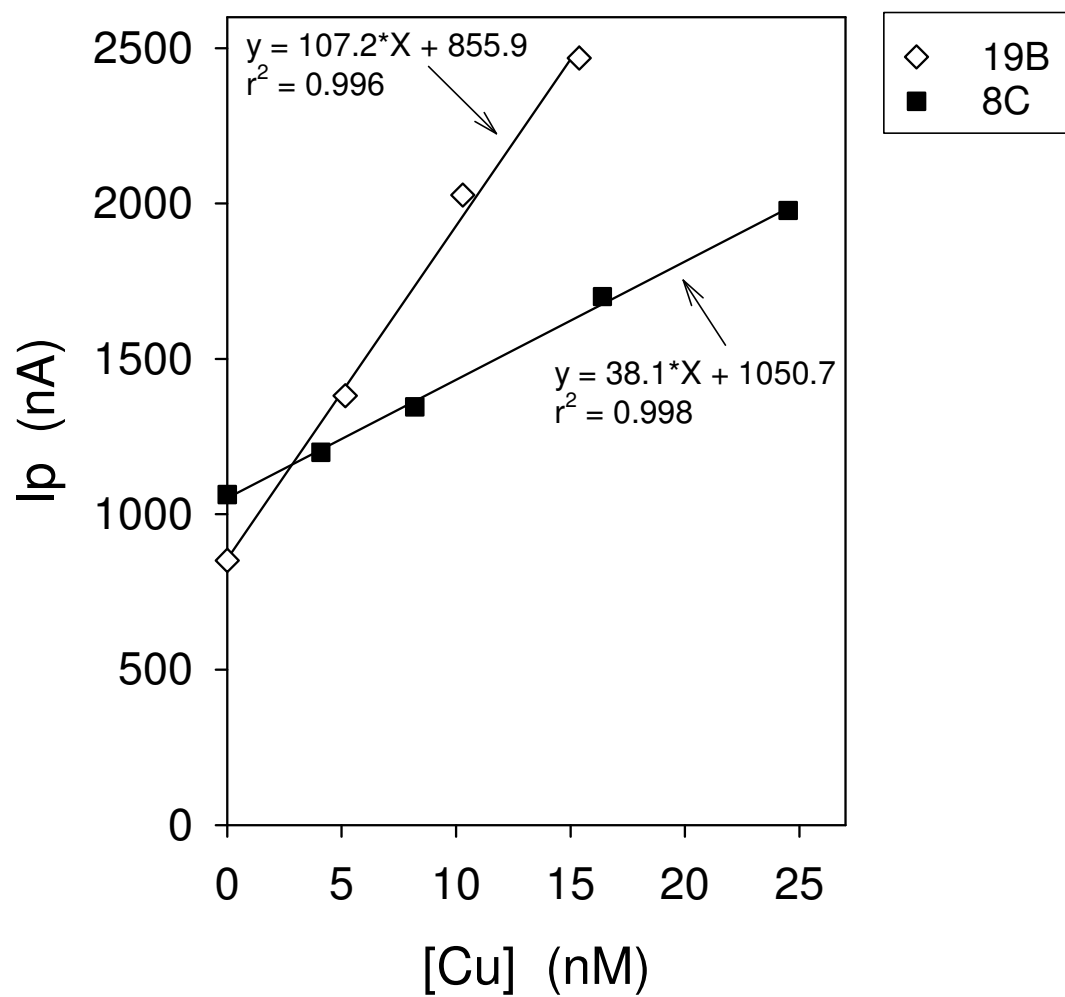


Figure 2

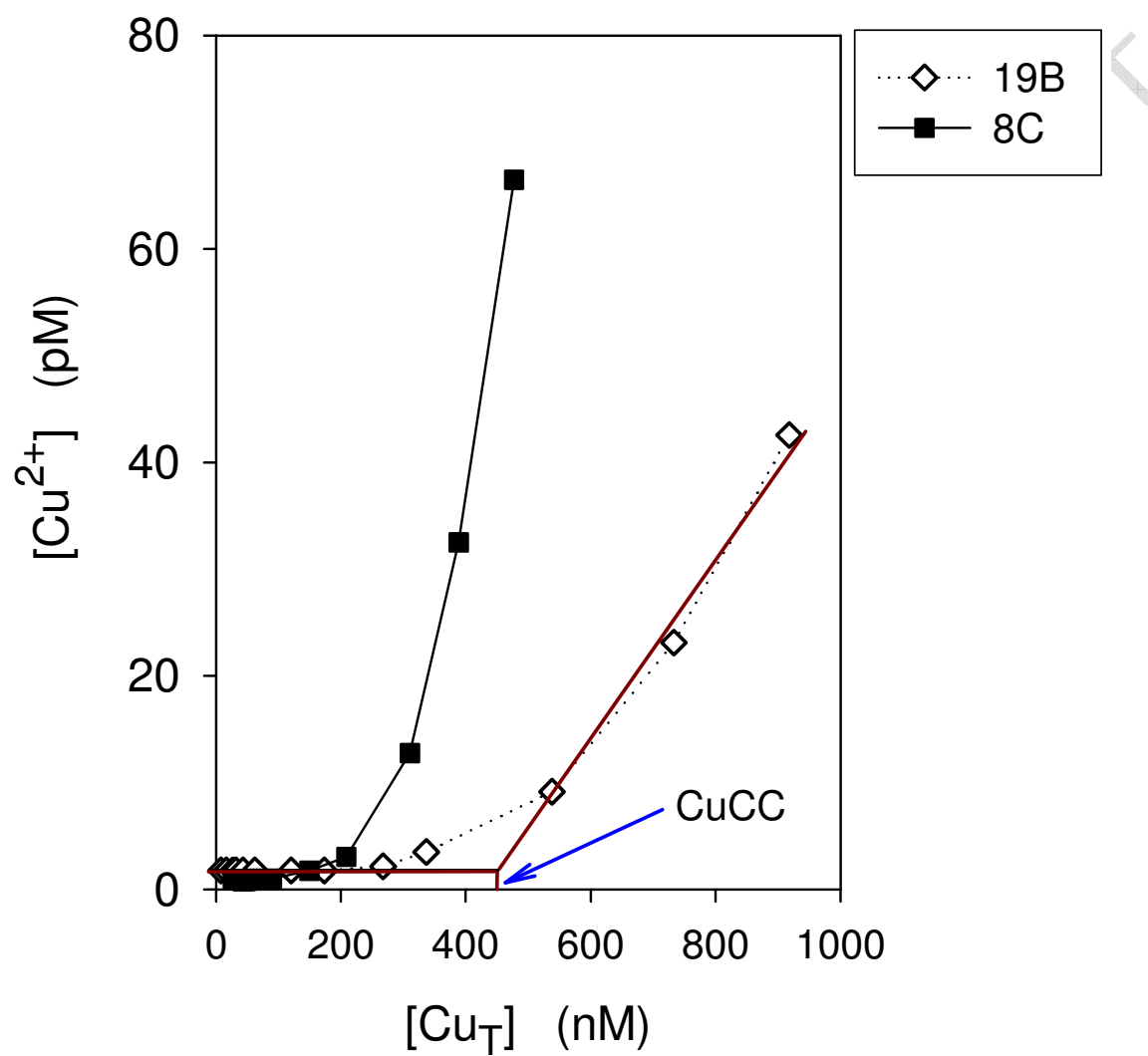


Figure 3

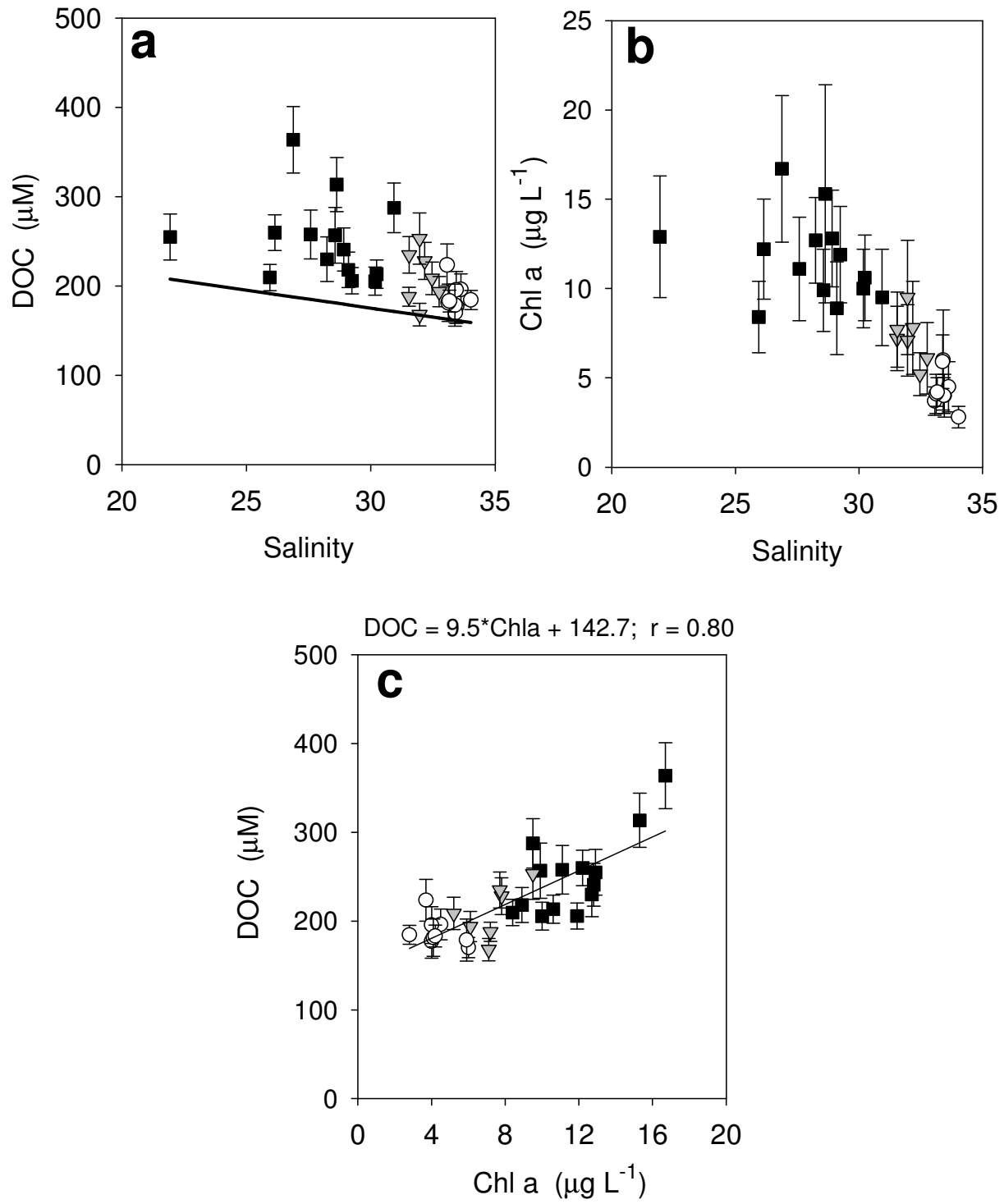


Figure 4

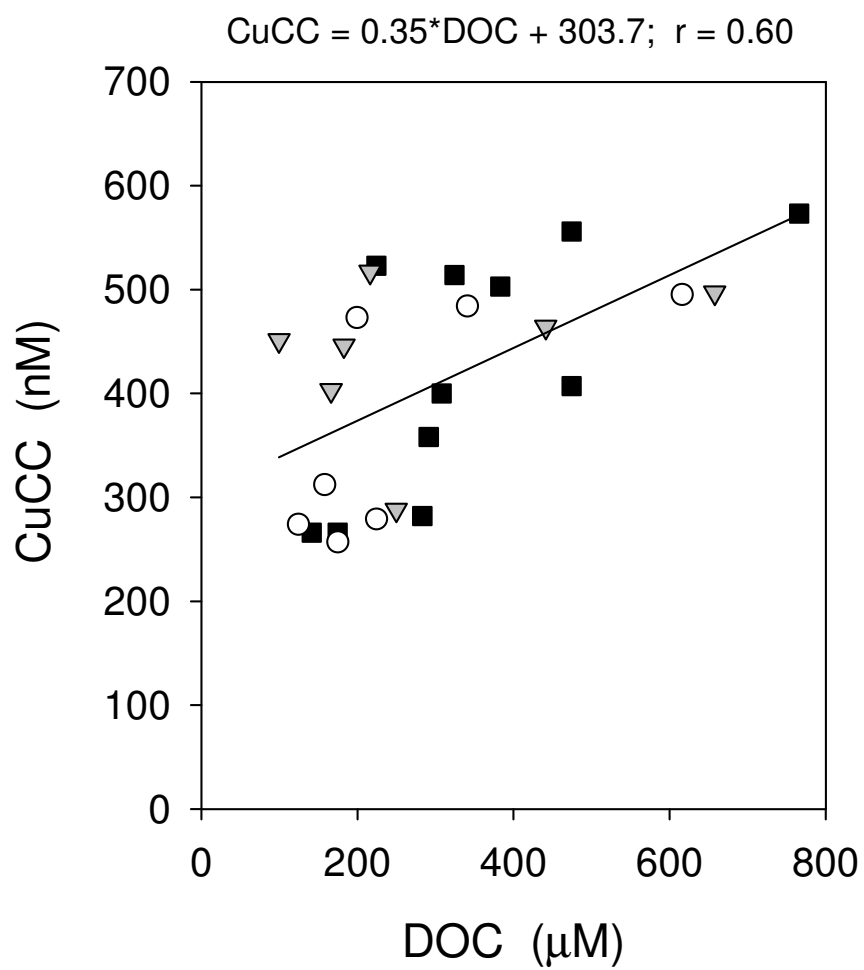


Figure 5