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Copper Toxicity to Larval Stages of Three Marine Invertebrates and Copper Complexation Capacity in San Diego Bay, California

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Temporal and spatial measurements of the toxicity (EC_{50}), chemical speciation, and complexation capacity (Cu-CC) of copper in waters from San Diego Bay suggest control of the Cu-CC over copper bioavailability. While spatial distributions of total copper (Cu_T) indicate an increase in concentration from the mouth toward the head of San Diego Bay, the distribution of aqueous free copper ion ($Cu(II)_{aq}$) shows the opposite trend. This suggests that the bioavailability of copper to organisms decreases toward the head of the bay, and is corroborated by the increase in the amount of copper needed to reach an EC_{50}, observed for larval stages of three marine invertebrates (Mediterranean mussel, *Mytilus galloprovincialis*, sand dollar, *Dendraster excentricus*, and purple sea urchin, *Strongylocentrotus purpuratus*), and by the increase in Cu-CC heading into the head of the bay. The amount of $Cu(II)_{aq}$ required to produce a 50% reduction in normal larval development (referred to here as pCu_{Tox}) of the mussel, the most sensitive of the three marine invertebrates, was generally at or above $\sim 1 \times 10^{-11}$ mol L^{-1} equivalents of Cu (i.e., $pCu_{Tox} \approx 11 = -(\log [Cu(II)_{aq}])$). These results suggest that the copper complexation capacity in San Diego Bay controls copper toxicity by keeping the concentration of $Cu(II)_{aq}$ at nontoxic levels.

Introduction

The complexation of copper in coastal marine environments restricts its availability to the biota (refs *1* and *2* and references therein). Total copper concentration ($[Cu_T]$) in marine environments includes that within the organisms themselves, the copper complexed with dissolved, colloidal, and particulate moieties, and the free aqueous copper ion (Cu^{2+} referred to here as $Cu(II)_{aq}$). Evidence indicates that an overwhelming fraction (i.e., >99.9%) of the dissolved copper in these environments is complexed by organic material and that the concentration of $Cu(II)_{aq}$ is 5 orders of magnitude lower than that of Cu_T (ref *3* and references therein). Despite this huge difference in concentration, $[Cu(II)_{aq}]$ reflects the availability of copper to organisms, making it a better predictor of its potential toxicity than $[Cu_T]$. This is suggested by the free-ion activity model (*4*) and substantiated by experimental evidence (*5*−*9*). However, as the partitioning among the chemical species of copper and the concentration of $Cu(II)_{aq}$ are regulated by both $[Cu_T]$ and the natural buffer capacity (i.e., the quantity of ligands, [L], available to bind copper, or the copper complexation capacity, Cu-CC) of the system, toxicity in marine environments is dependent on the copper buffering capacity.

To study the relationship between Cu-CC and copper toxicity, the effects of copper additions on larval development (EC_{50}) of the Mediterranean mussel (*Mytilus galloprovincialis*), sand dollar (*Dendraster excentricus*), and purple sea urchin (*Strongylocentrotus purpuratus*) were measured in seawater samples from San Diego Bay. These seawater samples represent, both spatially and temporally, the complete range of biogeochemical characteristics in the bay. The measured effects indicate a decrease in bioavailability of copper from the mouth to the head of the bay. This is in accordance with concentration distributions of $Cu(II)_{aq}$ and Cu-CC. Furthermore, the control of Cu-CC over copper bioavailability is further indicated by an observed steady-state (i.e., spatially and temporally constant) toxic threshold concentration of $Cu(II)_{aq}$, or pCu_{Tox}, in the bay of 1×10^{-11} equiv of Cu for mussel larvae.

Experimental Procedures

Sampling Setting and Composite Samples. Sampling was conducted as part of a whole-basin approach to measure and model the toxicity of copper in addition to the complete range of physical, biological, and chemical conditions in San Diego Bay. For the purposes of this effort, the bay was divided into 25 box segments along the axis of the bay; in addition, a box was included for both Shelter Island (box 6) and Commercial Basin (box 9) marinas (Figure 1). It should be noted that no data are presented here for these marinas, despite the fact that their station numbers are shown in several figures. Sampling events took place on Aug 30, 2000, Jan 30, 2001, May 11, 2001, Sept 19, 2001, Feb 27, 2002, and May 14, 2002. The Marine Environmental Survey Capability (MESC), aboard the Navy R/V Ecos, was used for the collection of the samples. Blake et al. (*10*) provides a more extensive discussion of these surveys. Composite samples were collected for each box segment with a flow-through system, by continuous pumping of the sample from approximately 2 m below the surface into 20 L carboys during the complete transit throughout each box. Subsamples from the composites were obtained for toxicity tests and the measurement of unfiltered copper concentration ($[Cu_T]$) and Cu-CC.

Toxicity Tests. Toxicity tests were conducted following guidance provided by the U.S. Environmental Protection Agency for estimating chronic toxicity to Pacific coast marine and estuarine organisms (*11*), using embryos of three invertebrate species. One bivalve, the Mediterranean mussel (*M. galloprovincialis*), and two echinoderms, the purple sea urchin (*S. purpuratus*) and the sand dollar (*D. excentricus*), were evaluated for normal larval development following exposures ranging from 48 to 96 h. The urchin and sand dollar were used interchangeably depending on the availability of gravid adults. Both echinoderm species were collected from clean areas near the mouth of Mission Bay in San Diego, CA, while mussels were from Carlsbad Aquafarm (Carlsbad, CA).

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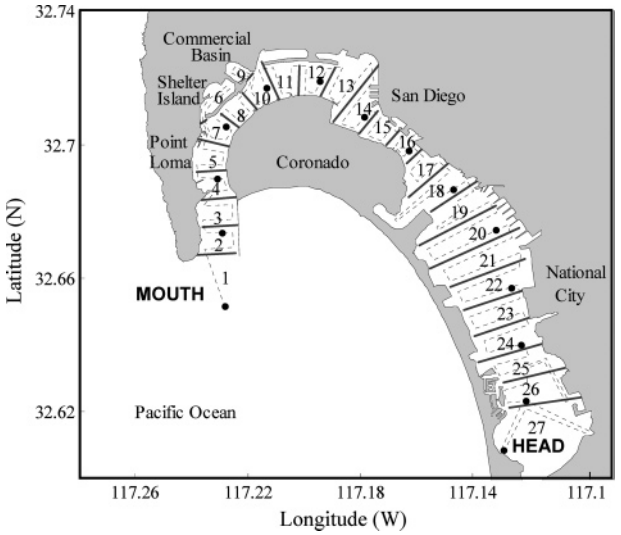

FIGURE 1. Description of the grid of boxes used for sampling in San Diego Bay. Each box is numbered, and the boundaries are shown. The position and number of Shelter Island and Commercial Basin are also provided. Discrete seawater samples were collected at the locations denoted with closed circles.

Gametes were obtained from echinoderms by injection of 0.5 M KCl into the gonads of approximately 10 individuals, while mussel spawning was induced by temperature shock of about 25 specimens. Within 4 h of fertilization approximately 200 embryos were added by pipetting the appropriate volume (e.g., 100 μL) of a counted embryo suspension to unfiltered seawater samples. Copper in these samples was allowed to equilibrate for approximately 1 h before the embryos were introduced. These samples included up to eight copper concentrations ranging from 0 to 80 μg L^{-1} (from 0 to 1.26 μM), by the addition of the necessary volume from a 1000 μg L^{-1} (15.7 μM) copper stock solution. Each treatment was replicated three times. Tests were conducted in 20 mL glass scintillation vials and held in a temperature-controlled light chamber at 15 °C with a 16 h light/8 h dark photoperiod. Water quality (temperature, pH, dissolved oxygen concentration, salinity) was monitored daily.

Tests were terminated with 10% buffered formalin following verification that controls had achieved the desired level of larval development. After 72−96 h, sea urchin and sand dollar embryos achieving normal development are pyramidal in shape and have four well-developed skeletal rods (pluteus). Normally developed bivalve larvae possess a hinged D-shaped shell within 48 h. Approximately 100 larvae were counted from each replicate at 40× magnification and scored as either normal or abnormal. Following confirmation of normal distribution and equality of variances with arcsine, square root transformed data, the proportion of normal larvae was used to compute EC_{50} values using Probit analyses with ToxCalc software (*12*). Copper concentrations used for EC_{50} calculations were based on nominal total copper additions from stock solutions measured by direct injection graphite furnace atomic absorption spectrometry (GFAAS). The initial total copper concentration unique to each seawater sample was added to the nominal concentrations.

Total Copper Concentration. Trace-metal clean techniques were used throughout the sampling, handling, and analysis of the samples. Samples for unfiltered (i.e., near-total) copper concentrations ($[Cu_T]$) were collected in 1 L acid-cleaned low-density polyethylene bottles and acidified to pH ≤ 2 with ULTREX-grade nitric acid in a class-100 all-polypropylene working area. Following a 10-week period to allow for the oxidation of organic matter, the samples were concentrated according to the APDC/DDDC liquid/liquid procedure detailed by Bruland et al (*13*). The efficiency of the concentration procedure for copper averaged 99% ± 8.7% (average ± 1 standard deviation), n= 18, for CASS-3 and CASS-4 of the National Research Council of Canada and 102% ± 8.6%, n = 4, for Standard Reference Material 1643d of the National Institute of Standards & Technology.

The concentration of copper in the preconcentrates was measured by GFAAS with stabilized platform techniques, Zeeman background correction, and the method of standard additions. The coefficient of variation of replicate measurements was ≤5%. Procedural blanks of high-purity (18 MΩ cm^{-1}) water had a copper concentration of 0.0042 ± 0.0019 μg L^{-1} (0.066 ± 0.030 nM). The method limit of detection, defined as 3 times the standard deviation of the procedural blanks, was 0.0056 ± 0.002 μg L^{-1} (0.089 ± 0.03 nM) for copper.

$[Cu(II)_{aq}]$ (pCu) and Cu-CC. The concentration of the free aqueous copper ion ($[Cu(II)_{aq}]$) was measured with an Orion 94-29 Cu(II) ion selective electrode (Cu-ISE), following procedures used by Zirino et al. (*14*), and Cu-CC was measured as detailed in Rivera-Duarte and Zirino (*15*); however, a brief description of the procedures is provided here. Both measurements were made in a dark, class 100 working station, with constant stirring at 25 ± 0.1 °C, by the electrode potential (mV) between a Cu-ISE and an Orion Ag/AgCl double-junction reference electrode. The electrodes were calibrated with seawater Cu-activity buffers made with 2×10^{-4} M Cu in filtered (0.45 μm) seawater and either 1×10^{-3} M ethylenediamine or 1×10^{-3} M glycine (*14*, *16*). Since $[Cu(II)_{aq}]$ in each buffer was calculated with a specific ion-interaction model for the measured pH and the concentrations of major ions (*16*), the calibrated response of the Cu-ISE is reported as the pCu (i.e., $-(\log [Cu(II)_{aq}])$) of the solution. The range in pCu for these calibrations was 13.0 ± 0.16 ($[Cu(II)_{aq}] \approx 1 \times 10^{-13.0}$ M) to 9.5 ± 0.16 ($[Cu(II)_{aq}] \approx 1 \times 10^{-9.5}$ M), n = 87, and while it covers both the largest values of pCu and the values of pCu at the Cu-CC reported here, it does not extend to the lowest values of pCu (~8, $[Cu(II)_{aq}] \approx 1 \times 10^{-8}$ M) measured in the titration. However, Belli and Zirino (*16*) demonstrated a linear response for the Cu-ISE and these buffers to $Cu(II)_{aq}$ concentrations up to 1×10^{-7} M.

The change in the response of the Cu-ISE during a titration with copper was used for the measurement of the Cu-CC (*15*). The titrations were performed with a TTT 85 titrator and an ABU 80 autoburet, both from Radiometer Copenhagen, connected to a personal computer for continuous automatic recording of the data. The measurement is performed as follows: first, the electrodes are calibrated and then allowed to equilibrate overnight in an aliquot of the seawater sample. The next day, an aliquot of 250−300 g of fresh seawater sample is weighed into a Teflon beaker, and the electrodes are allowed to equilibrate in it for several minutes before the titration is started. The titration then proceeds automatically by additions of 10 μL each once the potential has stabilized to within 0.1 mV s^{-1} and is complete after 99 mL of the titrant is added (equivalent to an average change in concentration of 7.6×10^{-7} M (48.2 μg L^{-1}, n = 78)). The titrant is made with 200 μL of 1000 ± 3 μg mL^{-1} high-purity copper standard added to 1 L of 18 MΩ water containing 32 g of NaCl. The Cu-CC is estimated from the inflection point of the resulting titration curve using a MATLAB routine (*15*).

Cu-CC by Differential Pulse Anodic Stripping Voltammetry (DPASV). Complementary determinations of the ligand concentration ([L] = Cu-CC) were carried out with DPASV according to ref *3*. For this determination, 25 mL aliquots of unfiltered sample were dispensed into a series of acid-cleaned polycarbonate vials by weight, to which copper was added at nominal concentrations of 0, 1, 2, 4, 6, 8, 10, 12, 16, 20,

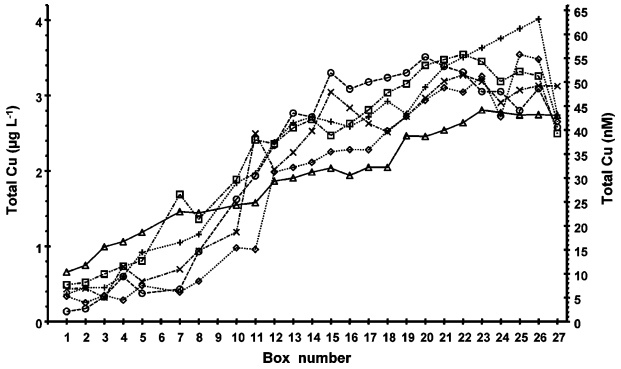

FIGURE 2. Distributions of total copper (Cu_T) for the six sampling events conducted in this study. The symbols for each cruise are Aug 30, 2000 (×), Jan 30, 2001 (□), May 11, 2001 (△), Sept 19, 2001 (◇), Feb 27, 2002 (○), and May 14, 2002 (+). Concentration units are provided as both μg L^{-1} and nM.

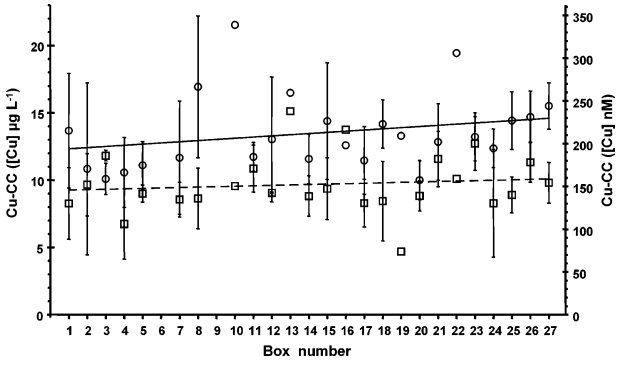

FIGURE 3. Spatial and temporal distributions of complexation capacity (Cu-CC or [L]) in San Diego Bay. Complexation capacities were measured by either a Cu-ISE (○) or DPASV (□). For the sake of simplicity, the data are presented as the mean value at a station for all survey events ± 1 standard deviation. Standard deviations are shown as continuous lines (—) unless $n = 1$. The figure also includes the linear regression for all the data collected by the Cu-ISE (—) and by DPASV (---). Concentration units are provided as both μg L^{-1} and nM.

and 24 μg L^{-1} (approximately 0, 16, 32, 63, 94, 126, 157, 189, 252, 315, and 472 nM, respectively). After an overnight equilibration period, the concentration of labile copper was determined with a thin mercury film (TMF) on a rotating glassy carbon disk electrode (RGCDE). Determinations were made in a class 100 working area with a BAS100 electrochemical analyzer, a Pine MSRX speed control and a Pine analytical rotator. The analysis was performed at a rotation speed of 4000 rpm, the sample was deposited for 315 s at −550 mV, and the analyte was stripped from −1000 to −100 mV in the Osteryoung square wave stripping voltammetry mode, with a sensitivity of 1×10^{-5} A V^{-1}. The areas of the peaks located at −300 mV were fed into a MATLAB routine for the calculation of [L] (i.e., Cu-CC).

Results and Discussion

Spatial and Temporal Distributions. In San Diego Bay, copper concentration gradients are essentially at steady state. An extensive discussion of the distributions of total copper and other parameters corresponding to our sampling events is provided by Blake et al. (*10*), and only a brief description is provided here. There is evidence for two temporal regimes driven by salinity within San Diego Bay: predominant hypersaline conditions and sporadic weak-estuarine conditions. Hypersaline conditions are associated with evaporation in the head of the bay and persist throughout most of the annual cycle, while weak-estuarine conditions were observed only after strong winter rainfall events (i.e., Jan 30, 2001, two weeks after a rainfall event). In addition to the presence of these temporal regimes, distributions of total copper (Cu_T) also indicate that three spatial regimes exist within the bay (Figure 2). An area with the lowest Cu_T concentrations is located from the mouth to the middle of the bay (boxes 1−11), an area with the highest concentrations to the south (boxes 12−24), and an area with decreasing Cu_T concentrations in the head of the bay (boxes 25−27). Despite the presence of these temporal and spatial regimes, the distribution of Cu_T concentrations remained relatively constant throughout the study (Figure 2), with an average change of concentration within each box of 0.94 ± 0.26 μg L^{-1} (15 ± 4.1 nM). The long-term persistence of the temporal and spatial distributions of Cu_T concentrations in San Diego Bay is further confirmed by the agreement of these measurements with those of Zirino et al. (*17*), Flegal and Sañudo-Wilhelmy (*18*), and Esser and Volpe (*19*). This steady-state condition with Cu_T concentrations increasing heading into the head of the bay could suggest a concurrent increase in bioavailability; however, the actual conditions observed in San Diego Bay are just the opposite.

The values of Cu-CC measured using the Cu-ISE and those measured by DPASV provide ligand concentrations of the same order of magnitude, but not identical values. Measurements of Cu-CC with DPASV had an overall range of 3.9−15.1 μg L^{-1} (62−238 nM), with an average and standard deviation of 9.5 ± 2.4 μg L^{-1} (150 ± 38 nM), and the corresponding values measured with the Cu-ISE are 6.2−21.5 μg L^{-1} (98−339 nM) and 13.1 ± 3.3 μg L^{-1} (206 ± 51 nM). As expected, determinations by DPASV in general provided lower values for Cu-CC than those made by the Cu-ISE. However, the agreement on the magnitude of the values measured with both techniques is excellent, as is demonstrated by the spatial and temporal overlapping found for these measurements in this study (Figure 3). The difference in the values measured is expected from the different detection windows and concentration working ranges of these techniques (*15*, *20*). The measurements with the Cu-ISE also indicate an increase in complexation capacity heading into the bay (Figure 3). This is calculated from the regression analysis of all of the data obtained in this effort, which results in a regression equation of $0.091x + 11.7$ ($n = 72$). The slope of this regression is different from zero at a 95% confidence level, while that from DPASV analysis ($0.061x + 8.66$, $n = 69$) is not. This indicates that the complexing material that drives Cu-CC in the inner parts of the bay should be made up of relatively weaker ligands, as these are not detected by DPASV. The increase in Cu-CC observed for the Cu-ISE measurements suggests a decrease in the concentration of $Cu(II)_{aq}$ heading into the head of the bay, which was indeed observed throughout most of the sampling events (*10*). Thus, despite the increasing levels of Cu_T into the bay, the increase in Cu-CC determines the distribution of $Cu(II)_{aq}$ observed in the bay.

Complexation capacity varied substantially more than [Cu_T], both spatially and temporally (Figure 3). There was significant within-station variation, with an average change in Cu-CC in each box of 5.1 ± 2.7 μg L^{-1} (∼80 ± 43 nM) for the Cu-ISE measurement, and of 3.5 ± 1.7 μg L^{-1} (∼55 ± 27 nM) for DPASV measurements. There also was some temporal variation with lower complexation observed on Jan 30, 2001, and the greatest complexation on May 11, 2001, and May 14, 2002 (data not shown).

With respect to toxicity, EC_{50} (the concentration at which 50% of the larvae did not develop normally compared to the control) values also increased heading into the bay (Figure 4). This trend indicates that heading into the bay, more copper was required to elicit the same toxic effect on the test

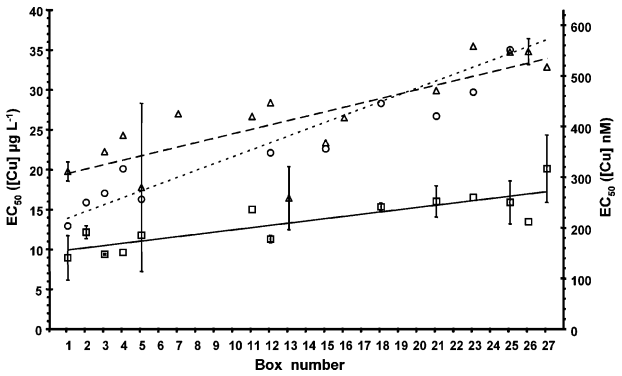

FIGURE 4. Spatial and temporal distributions of the concentration that reduces the development of larvae by 50% with respect to the control (EC_{50}). For the sake of simplicity, the data are presented as the mean value at a station for all survey events ± 1 standard deviation (—). The symbols for each species of marine invertebrates are Mediterranean mussel (*M. galloprovincialis*) (□), purple sea urchin (*S. purpuratus*) (○), and sand dollar (*D. excentricus*) (△). The figure also includes the linear trends for the mussel ($0.28x + 9.65$, $r = 0.846$ (—)), purple sea urchin ($0.86x + 13.00$, $r = 0.930$ (---)), and sand dollar ($0.55x + 18.95$, $r = 0.801$ (— —)). Concentration units are provided as both μg L^{-1} and nM.

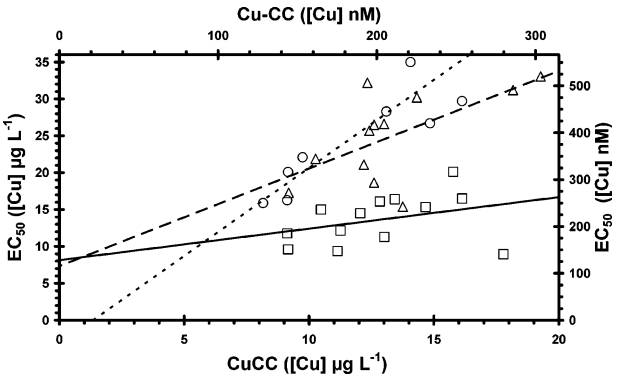

FIGURE 5. Control of toxicity (EC_{50}) by copper complexation capacity (Cu-CC). The data in this plot are given as the mean of the values measured for each species at each station for all surveys. The symbols for each species of marine invertebrates are Mediterranean mussel (*M. galloprovincialis*) (□), purple sea urchin (*S. purpuratus*) (○), and sand dollar (*D. excentricus*) (△). The linear trends for the mussel ($0.43x + 7.94$, $r = 0.349$ (—)), purple sea urchin ($2.39x - 3.24$, $r = 0.904$ (---)), and sand dollar ($1.32x + 7.32$, $r = 0.627$ (— —)) are also included in the figure. Concentration units are provided as both μg L^{-1} and nM.

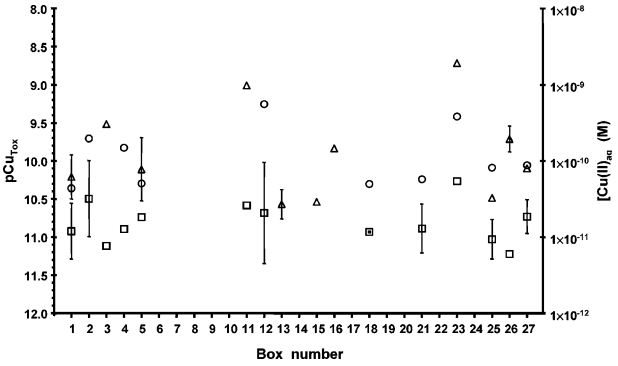

FIGURE 6. Threshold toxic concentration (pCu_{Tox}) of the free copper ion ($Cu(II)_{aq}$). The values of pCu_{Tox} are given as the mean ± 1 standard deviation for all the toxicity tests at each box. Measured values are provided for those boxes where there was only one toxicity test. The symbols for each species of marine invertebrates are Mediterranean mussel (*M. galloprovincialis*) (□), purple sea urchin (*S. purpuratus*) (○), and sand dollar (*D. excentricus*) (△). Concentration units are provided as both pCu (−(log [$Cu(II)_{aq}$])) and M.

organisms. The degree of response to copper additions differed among the three species, with sensitivity decreasing in the following order: mussel, sea urchin, and sand dollar (Figure 4). Some temporal variation within stations was observed, which seems to be more significant for the sand dollar (Figure 4). Despite this difference in sensitivity, all three species responded with increasing EC_{50} values toward the head of the bay. This increase in EC_{50} indicates a decrease in copper bioavailability heading into the head of the bay, in concordance with the distribution of Cu-CC (Figure 3) but in discordance with that for Cu_T (Figure 2). Temporal variability in EC_{50} values was also observed, with lower values for Jan 30 and May 11, 2001, in contrast to the other sampling events (data not shown).

Control of Cu-CC by Particles. While total copper concentrations remained relatively stable, there was a spatial and temporal variation in complexation capacity in San Diego Bay. This could be explained by the variability of the assortment of moieties in the system following the free-ion activity model. For example, as mentioned above, Cu-CC was lowest on Jan 30, 2001. The water in the bay at this time was extremely clear, which was associated with the lowest measurements in the concentration of total suspended solids (TSS, 0.3−2 mg L^{-1}); in contrast, concentrations of chlorophyll α for this sampling event were within normal range (*10*). These conditions suggest that particles may be important in controlling Cu-CC. Despite the fact that TSS concentrations in the bay are usually low (≤7.5 mg L^{-1}), a decrease in their concentration was associated with a decrease in Cu-CC and the highest concentrations of $Cu(II)_{aq}$ measured throughout the study (*10*), even though biological parameters remained relatively stable on Jan 30, 2001.

Control of EC_{50} by Cu-CC. The bioavailability of copper in San Diego Bay appears to be affected by the Cu-CC. EC_{50} values and the Cu-CC measured with the Cu-ISE follow similar spatial patterns, as both parameters show a general increase into the head of the bay. The similarity in their distributions is substantiated by the covariance between these parameters (Figure 5). Regression analyses for specific sampling events and organisms indicate correlation coefficients that are significant at the 95% level (i.e., mussel on Feb 27, 2002, $r = 0.913$; sea urchin on Feb 27, 2002, $r = 0.906$). This correlation supports the association of Cu-CC with the EC_{50}. The difference in sensitivity among species is further illustrated in Figure 5, as both the sea urchin and sand dollar required a larger increase in copper (i.e., higher EC_{50} values) than the mussel to reach the same toxic effect.

Toxic Concentration of $Cu(II)_{aq}$ and Cu-CC. The bioassays determined that the concentration of $Cu(II)_{aq}$ that is toxic to the mussel larvae (pCu_{Tox}) in general was at or above 1×10^{-11} M (pCu 11) (Figure 6). This toxic concentration, pCu_{Tox}, is the $[Cu(II)_{aq}]$ at the EC_{50}, and is estimated from the EC_{50} (i.e., $[Cu_T]$) measured for the larvae and the $[Cu(II)_{aq}]$ measured with the Cu-ISE at the EC_{50} on the corresponding seawater sample. This value was largely determined by the mussel larvae, with the mussel being more sensitive than either the sea urchin or the sand dollar (Figure 6). While pCu_{Tox} showed some temporal variability, with values for the mussel within one station changing by up to 1 order of magnitude, the minimal value of pCu_{Tox} remained relatively constant throughout the study. The toxic threshold for most of the sampling cruises occurred at a pCu ≈ 11.0 ($[Cu(II)_{aq}] \approx 1 \times 10^{-11}$ M; Figure 6). This may be an environmental parameter that can be useful for the prediction of toxic effects of copper in coastal embayments. The spatial and temporal distributions of the copper species presented herein support the notion that copper bioavailability is related to and possibly

controlled by the complexation capacity. This is achieved by keeping the concentration of $Cu(II)_{aq}$ below toxic levels, despite the diverse set of environmental conditions observed in San Diego Bay, including the general increase in $[Cu_T]$ from the mouth to the head of the bay.

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